ATYPICAL PROTEIN KINASE C ISOFORMS IN DISORDERS OF THE NERVOUS SYSTEM AND CANCER

FIELD OF INVENTION

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The present invention relates to atypical isoforms of protein kinase C (aPKC) and their role in the intracellular mislocalization of gene products in nervous system disorders. In particular, the present invention relates to the discovery that aPKC isoforms are altered in neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD). The present invention is further directed to the use of aPKC in diagnosis, drug screening and treatments, including gene therapy, for neurological and psychiatric disorders.

The present invention also relates to the atypical isoforms of protein kinase C and their role in cancer. In particular, the present invention relates to the discovery that the atypical PKCζ gene is commonly altered in numerous forms of cancer. The present invention is further directed to the use of aPKC in diagnosis, treatment and development of treatments for cancer.

BACKGROUND OF INVENTION

Protein kinase C (PKC) consists of a heterogeneous family of isozymes derived from nine genes divided into three classes: conventional, novel and atypical. The atypical class of isoforms (aPKC), e.g., PKCζ (PKCζI/II) and PKCι/λ, is distinguished from the conventional and novel classes by their insensitivity to calcium and diacylglycerol (DAG), the classical activators of PKC (Newton, 2003, Biochem J, 361-71). Together with the proteins encoded by par3 and par6 genes, aPKC has been shown to play a critical role in cell polarity (Ohno, 2001, Curr Opin Cell Biol, 13(5): 641-48). Recently, the par3 and par6 genes were also shown to play a role in the formation of neuronal axons (Shi et al., 2003, Cell, 112(1): 63-75). Loss of function of any of par3, par6 and aPKC proteins can result

in disruption of cell polarity.

In addition, a role for aPKC in synaptic plasticity, learning and memory has been established (Sacktor et al., 1993, Proc Natl Acad Sci USA 90:8342-46; Osten et al, 1996, Neurosci Letter 221:37-40; Drier et al, 2002, Nat Neurosci 5:316-24; Ling et al, 2002, Neurosci 5:295-96). In long-term potentiation (LTP), a widely studied model for memory, aPKC plays an important role in the two distinct temporal phases. While full-length aPKC forms are activated during the LTP induction phase, a truncated form of aPKC, termed PKMζ (i.e., PKCζII), is activated during the maintenance phase of LTP. PKMζ is identical to PKCζI, except that it lacks an autoinhibitory regulatory domain (Hernandez, et al., 2003, J Biol Chem 278, 40305-16 and Hirai et al., 2003, Neurosci Lett 348, 151-54). Although all of the PKC isoforms can theoretically have PKM forms (i.e., truncated or independent catalytic domains of PKC), the only PKM form that is consistently observed in the normal brain is PKMζ (Sacktor et al., 1993). Recent work has shown that PKMζ is both necessary and sufficient for long-term potentiation (LTP) maintenance (Ling et al). Furthermore, expression of PKMζ prolongs memory in an odor avoidance task in *Drosophila melanogaster* (Drier et al). Thus, the role of PKMζ in memory appears conserved in widely divergent species. Consistent with this notion, the human form of the PKMζ mRNA has been identified (Hernandez, et al).

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive impairment in memory and cognitive functions. Imbalances in neuronal signal transduction pathways have been implicated in AD's pathogenesis (Shimohama et al., 1990, J Neural Transm Suppl 30:69-78). Several studies have demonstrated abnormalities of protein kinase C (PKC) function in brain tissue of AD patients (Cole et al., 1988, Brain Res 452:165-174; Clark et al., 1991, Lab Invest 64, 35-44; Horsburgh et al., 1991, J Neurochem 56:1121-1129; Masliah et al., 1990, J Neurosci 10:2113-24; Masliah et al., 1991, J Neurosci 11:2759-2767; Saitoh et al., 1990, Adv Exp Med Biol 265:301-10; Saitoh et al., 1993, Acad Sci 695:34-41; Shimohama et al., 1993, Neurology 43:1407-1413; Lanius et al., 1997, Brain Res 764:75-80; Wang et al., 1994, Neurobiol Aging 15:293-298).

Microscopically, the two major features of AD are the presence of β-amyloid (A β) containing senile plaques (SP) and tau containing neurofibrillary

tangles (NFT). Amyloid angiopathy, defined as amyloid deposition in blood vessels, also occurs to a varying extent. Other changes include granulovacuolar degeneration (GVD) and Hirano body (HB) formation.

The NFT are abnormal structures, composed mainly of paired helical filaments (PHFs) consisting of a hyperphosphorylated form of the microtubule-associated protein tau (Buee et al., 2000, *Brain Res Brain Res Rev* 33, 95-130). The distribution of tau is largely restricted to axons, where it functions mainly to stabilize microtubules (MT) and promote MT polymerization. In NFT-containing neurons, however, tau-associated PHFs can be found throughout the cytoplasm and dendrites. Other components of the NFT include microtubule-associated protein 2 (MAP2) and ubiquitin. A "ghost tangle" is a type of NFT, where the surrounding neuron has completely degenerated. Ghost tangles can contain deposits of Aβ that accumulate after cell death. PHFs also exist in dystrophic neurites surrounding the senile plaques as well as in other neurites, where they are termed neuropil threads.

In addition to AD, at least 20 different diseases have tau-based neurofibrillary pathology as a feature and are collectively known as tauopathies (Lee et al., 2001, *Annu Rev Neurosci* 24, 1121-59). This group includes AD, Pick's disease (PiD), frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). Considerable heterogeneity exists between the tangles found in the various tauopathies. For example, NFT in AD are composed principally of PHFs with a diameter of 8-20 nm; some straight filaments are also seen in AD. In contrast, the tangles of FTDP-17 are twisted and straight, but not paired and helical. Nevertheless, the tau protein in all these disorders is hyperphosphorylated.

It has been argued that tau-based tangle formation is not the primary pathological event in AD since tangles appear in a number of other disorders. This apparent lack of specificity suggests that the NFT is a final endpoint of a number of pathophysiologic processes rather than an initiating event. Contrary to this view, it has been discovered that a mutation in the tau gene itself in multiple FTDP-17 family lineages can lead to tau dysfunction and degeneration in the absence of Aβ accumulation (Lee et al., 2001).

NFT develop through stages (Braak et al., 1994, *Acta Neuropathol* 87, 554-67). The earliest form of NFT is the pre-NFT, where PHFs begin to form,

but the full tangle has not yet developed. The next is the intracellular stage, where the cytoplasm becomes filled with hyperphosphorylated tau. The final stage is the "extracellular" NFT, where cell death has occurred, the membrane and organelles cleared away, but the cytoskeletal remnants remain. The final stage is also referred to as "ghost" tangles. Using phosphospecific tau antibodies, it was discovered that the pattern of tau phosphorylation varies between these three different stages (Augustinack et al., 2002, *Acta Neuropathol (Berl)* 103, 26-35). Theoretically, if one knows which residues on tau are phosphorylated first, then one can have a better chance at identifying the kinase responsible for the tau phosphorylation. It was demonstrated that T153, S262 and T231 were among the first residues in tau to become phosphorylated stages (Augustinack et al., 2002).

Despite extensive research, the particular kinase that phosphorylates tau in AD has not been clearly identified (Buee et al., 2000). The longest tau protein isoform contains 79 possible serines and threonines, with phosphorylation of least 30 of them occurring in AD. Numerous kinases have been shown to phosphorylate tau *in vitro*, but leading candidate kinases have been shown to phosphorylate tau *in vivo*. These are glycogen synthase kinase 3β (GSK3β) and cyclin-dependent kinase 5 (cdk5). Both are members of a group of proline-directed kinases that prefer serine/threonine residues directly followed by a proline. Abnormal tau from an AD brain contains a mixture of hyperphosphorylated serine and threonine residues with and without a trailing proline. Indeed, researchers have postulated multiple kinases phophorylating tau at different sites, which can be proline directed and non-proline directed protein kinases.

The conventional PKCα isoform can phosphorylate the cytoskeletal protein tau. It is not the full-length form of PKCα, but the truncated PKMα form of the enzyme which is selectively capable of phosphorylating tau (Cressman et al., 1995, M. *FEBS Letter* 367, 223-27). The only PKM isoform that is consistently overserved in the brain is PKMζ (Naik et al., 2000, J Comp Neurol., 426(2):243-58).

The present invention, for the first time, shows that PKMζ directly phosphorylates tau. For this reason, increases in aPKC will cause NFT formation. Furthermore, aPKC phosphorylates GSK3β, a kinase well known to phosphorylate tau, leading to inactivation of GSK3β (Lavoie et al., 1999, *J Biol Chem* 274, 28279-85). For this reason, decreases in aPKC activity constitute a removal of the negative

regulation of GSK3β activity and indirectly causes NFT formation. The present invention provides that apkc and gsk3b, both known regulators of cell polarity, are present in a complex. Alterations in the signaling or activity of these two kinases leads to mislocalization of gene products within the intracellular and extracellular compartments as well as abnormal posttranslational modifications of gene products and subsequent pathology including neurological dysfunction and cancer.

While the etiology of cancer is heterogenous, and largely depends on the tissue type, there is a consensus among those skilled in the art that human cancer cells show a markedly increased genetic mutation rate (genetic instability). The mutations can manifest themselves as chromosomal abnormalities (e.g. deletions, insertions, amplifications, mutations and rearrangements) and point mutations. The effects of such genomic alterations are varied; but some of these alterations are direct contributors to cancer development. Cancer develops through stages. The early stages tend to be more benign, with a better prognosis. In contrast, the later stages are more malignant, and tend to metastasize to a larger extent. Late stage cancers tend to have accumulated more chromosomal alterations. The present invention utilizes alterations in the aPKC genes and their RNA or protein products to diagnose, stage, treat, and develop treatments for cancer.

One example of a chromosomal alteration associated with cancer development is deletion of the short arm of chromosome 1 (1p). Chromosome 1p deletions have been observed in a wide variety of cancers. It has been estimated that over one half of solid tumors are associated with chromosome 1p deletions. The current method for determining the status of chromosome 1p in tumors involves fluorescence in situ hybridization (FISH). While highly sensitive, FISH has numerous drawbacks including the fact that it is not routinely used in pathology laboratories, is costly and time consuming. The present invention provides a new way to determine the status of chromosome 1p in tumors using probes to the PKCζ gene, such as antisera. Antisera based methods are routinely used in pathology laboratories, are robust and inexpensive. For this reason, PKCζ gene status is by far superior to FISH in determining cancer status.

The PKCζ gene lies on the short arm of the first chromosome (1p36.33). The gene lies a relatively short distance, about 2 million base pairs, away from the telomere. The proximity of the PKCζ gene to the telomere should

theoretically make it susceptible to chromosomal deletion and rearrangement. It is demonstrated by the present invention that the presence or absence of aPKC in cancers is useful for their diagnosis and staging.

The effect of deletion of chromosome 1p varies depending on the tumor type. For example, deletion of 1p in oligodendroglioma is a favorable prognostic indicator. In contrast, chromosome 1p deletion in neuroblastoma is considered unfavorable. As demonstrated by the present invention, one skilled in the art can now determine the status of chromosome 1p using specific probes to PKCζ. Examples of such probes include antisera, complimentary DNA or RNA sequences to aPKC, or PCR primers. The presence or absence of PKCζ is effective in determining the variety, stage or grade of the tumor.

SUMMARY OF THE INVENTION

The present invention demonstrates that an increase or a decrease in aPKC activity can lead to the development of pathological changes in cells, leading to nervous system dysfunction and cancer. It is contemplated by the presented invention that alteration in aPKC signaling contributes to the pathogenesis of nervous system disorders, such as Alzheimer Disease (AD). Accordingly, the localization of aPKC in NFT and the fact that disruption in aPKC signaling causes tau phosphorylation establish that aPKC alterations participate in pathogenesis of nervous system disorders. The present invention thus provides aPKC as a novel target for rational drug design useful for modulating aPKC activity for the purpose of treating nervous system disorders. The present invention further provides methods for diagnosis of nervous system disorders.

Specifically, it has been discovered by the present invention that PKMζ, a key molecule in the maintenance of long-term potentiation (LTP) and memory formation, e.g., in *Drosophila*, colocalizes with the NFT. Accordingly, disruption in PKMζ signaling contributes to the pathogenesis of nervous system disorders, such as AD. The presence of PKMζ protein in the human brain is also demonstrated by the present invention.

In accordance with the present invention, one aspect of the present invention is the identification of atypical isoforms of protein kinase C (aPKC), e.g. PKMζ, and their role in nervous system disorders, such as AD.

Another aspect of the present invention provides a method for

diagnosing nervous system disorders, such as AD. According to the present invention, a method for diagnosing neurological dysfunctions or nervous system disorders in a subject, using an aPKC specific probe, is provided. The diagnostic method of the present invention includes the steps of:

a. contacting a sample from the subject, e.g. a tissue biopsy, cerebrospinal fluid (CSF), or a blood sample, with an aPKC specific probe,

b. detecting the binding of the probe to the sample to determine the amount, localization or activity of the aPKC in the sample,

c. contacting a control sample with the aPKC specific probe,

d. detecting the binding of the probe to the control sample, e.g. a tissue biopsy, CSF, or a blood sample, to determine the amount, localization or activity of the aPKC in the control sample, and

e. comparing the amount, localization or activity of the aPKC in Step b with the amount, localization or activity of the aPKC in Step d, whereby if the amount, localization or activity of the aPKC in Step b is different from the amount, localization or activity of the aPKC in Step d, a nervous system disorder in the subject is present.

Still another aspect of the present invention provides methods for screening drugs or molecular compounds useful for modulating the amount or activity of the aPKC. According to the present invention, a method for screening molecular compounds, e.g. peptides and small molecules, using cells and aPKC specific probes is provided. Such method includes:

a. providing cells wherein aPKC gene is expressed,

b. incubating the cells with a candidate compound for a sufficient time to induce a change in the amount, localization or activity of aPKC in the cells,

c. incubating the cells as in Step b, in the absence of the candidate compound,

d. contacting the cells from Step b and Step c with an equal amount of an aPKC-specific probe,

e. detecting the binding of the probe to the cells to determine the amount, localization or activity of the aPKC in the cell from Step b and Step c,

and

f. comparing the amount, localization or activity of the aPKC in the cells, wherein if the amount, localization or activity of the aPKC in the cells from Step b is different from the amount, localization or activity of the aPKC in the cells from Step c, the candidate compound is identified as a compound that modulates aPKC.

An alternate method for screening molecular compounds for use in treatment of brain disorders involves the over-expression or deletion of genetic elements containing atypical PKC sequences in cells or animals, e.g. mice, and assaying said cells or animals for atypical PKC activity or levels.

In a further aspect of the present invention, an *in vitro* cell-free method for screening drugs or molecular compounds useful for modulating the amount, localization or activity of the aPKC, using aPKC and a substrate, is provided. Such method includes:

a. incubating an aPKC in a first container with adenosine triphosphate (ATP), preferably radioactively labeled ATP, a substrate of the aPKC, e.g. protein tau, with a candidate compound, for a sufficient time to induce a change in the amount or activity of the aPKC in the first container,

b. incubating the aPKC in a second container with ATP, preferably radioactively labeled ATP, and the substrate of said aPKC, e.g. protein tau, for a sufficient time to induce a change in the amount or activity of aPKC in the second container,

c. quantifying the amount of incorporation of the phosphate into the substrate in the first container and the second container, and

d. comparing the amount of incorporation in the first container and the second container, whereby a difference in the amount of incorporation in the first container and the second container inindicates that the candidate compound is identified as a compound modulating aPKC amount, localization or activity.

A variation of this method involves using a phospho-specific antisera to quantify phosphate incorporation into the substrate.

In still a further aspect of the present invention, methods, including

gene therapy, for treating and/or preventing neurological disorders are provided. According to the present invention, a method for treating and/or preventing neurological dysfunction is provided which employs a sequence corresponding to an aPKC, e.g. PKMζ (or an antisense sequence of aPKC) and an expression vector. This method includes the steps of:

a. inserting the aPKC sequence, e.g. PKMζ, into the expression vector, and

b. administering the vector to a subject, e.g. a patient,

whereby the vector treats and/or prevents a neurological dysfunction.

The methods of the present invention can be employed in the treatment and/or prevention of a variety of neurological disorders characterized by abnormal aPKC activity, such as phosphorylation of protein tau and co-localization with NFT. Such tau-associated filamentous aggregates are neuropathological hallmarks of Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD), Parkinson's Disease (PD), for example.

In yet a further aspect of the present invention provides antibodies against PKMζ for detection, treatment and/or prevention of neurological disorders, e.g. AD.

Thus, an object of the present invention is to detect, treat and/or prevent neurological disorders, such as Alzheimer's disease.

A further aspect of the present invention provides a method for constructing transgenic animals, such as a knock-out mouse lacking aPKC or a mouse that overexpresses the wild type or a mutant aPKC, that are useful as animal models of neurological dysfunction. The method includes:

a. constructing a transgenic animal having an altered aPKC amount, localization or activity, e.g., by deleting an aPKC gene in the animal using knock-out techniques known to one skilled in the art,
b. treating the transgenic animal from Step a with a candidate treatment process,
c. treating the transgenic animal from Step a with a control treatment process,
d. assaying the transgenic animals from Steps b and c for a biochemical or

behavioral change, and

e. comparing the results of the assay of the transgenic animals from Steps b and Step c, wherein a difference is indicative the efficacy of the candidate treatment process in alleviating the neurological dysfunction.

A variation of the method involves over-expression of either wild-type or mutated aPKC in a transgenic animal, alone or in combination with one or more other transgenes expressing mutations or polymorphisms in genes associated with neurodegeneration, e.g. superoxide dismutase, tau, β-amyloid, α-synuclein, and apolipoprotein E.

Still a further aspect of the present invention involves the genetic screening of DNA for mutations or polymorphisms in the DNA sequence of the aPKC genes, e.g. PKCζI/II and PKCι/λ, and regions regulating the expression of these genes including promoter regions, enhancer regions and negative regulatory regions. A method for such screening includes:

a. isolating DNA from a sample of a subject,

b. sequencing an aPKC gene or its regulating region of the DNA from Step a and,

c. comparing the DNA sequence from Step b to a reference DNA sequence, wherein the reference DNA is a known normal DNA sequence of the same region as sequenced DNA in Step b but without any mutation, whereby a difference between the DNA sequence from Step b and the reference sequence is indicative of genetic susceptibility to a neurological or psychiatric disorder.

A particular aspect of the present invention is directed to the use of a known aPKC interacting protein for all of the above methods.

In a further aspect, the present invention is directed toward the treatment of other abnormal protein aggregates including α-synuclein seen in PD, multisystem atrophy (MSA) and dementia with Lewy bodies (DLB) and β-amyloid in AD and normal aging.

In another aspect, the present invention provides methods for diagnosing various forms of cancer, such as neuroblastoma. According to the present invention, a method for diagnosing and/or staging a tumor is provided. The diagnostic method of the invention includes the steps of:

a. contacting an aPKC specific probe, preferably a PKCζ specific probe, with a tumor sample, e. g. a human biopsy sample, from a subject,
b. detecting the binding of the probe to the tumor sample to quantify the level, amount or activity of said aPKC in said tumor sample,
c. contacting the aPKC specific probe with a control sample,
d. detecting the binding of the probe to the control sample to quantify the level, amount or activity of the aPKC in the control sample, and
e. comparing the level, amount or activity of the aPKC in Step b with the level, amount or activity of the aPKC in Step d, wherein the difference of the level, amount or activity of the aPKC is indicative of the type and the staging of the tumor.

Still another aspect of the invention provides methods for drug screening for the purpose of treating and/or preventing cancer, e. g. neuroblastoma. According to the present invention, a method for screening molecular compounds, e. g. peptides and small molecules, is provided.

Yet another aspect of the present invention provides methods for treating cancer with gene therapy.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A illustrates that PKCζ phosphorylates the cytoskeletal protein tau. Autoradiogram showing incorporation of radioactive phosphate into tau Lane 1 shows that tau, in the absence of kinase, shows little incorporation of phosphate. Lane 2 and 4 show that in the absence of tau, there is no phosphorylarion. Lane 3 demonstrates that when tau is incubated with PKCζ phosphate is incorporated into tau. Lane 5 was included as a control, since GSK3β is well known to be able to phosphorylate tau. Figure 1B illustrates that cotransfection of PKMζ with GSK3β into neuroblastoma cells decreases tau phosphorylation. Western blot on protein extracts from neuroblastoma cells (IMR-32) transfected with full-length human tau and the indicated constructs were probed with a phosphospecific anti-tau (p396) antisera. Lane 1 shows that cotransfection of GSK3β results in a prominent degree of phosphorylation of tau on p396. Lane 2 shows that cotransfection with EGFP alone does not result in an increase in phosphorylated tau. Lane 3 shows that transfection with an EGFP-tagged kinase inactive mutant of PKMζ (EGFP-PKMζ-KI, K281W) also fails to result in increased

tau phosphorylation. Lane 4 shows that transfection of GSK3β and EGFP-tagged PKMζ results in less phosphorylation of tau than GSK3β alone (compare with lane 1). Lane 5 shows that EGFP-tagged PKMζ is capable of phosphorylating tau (compare with lane 2 and 3) but not to the same degree as GSK3β (compare with lane 1). Lane 6 shows that cotransfection of EGFP-PKMζ-KI with GSK3β also results in decreased tau phosphorylation (compare to lane 1).

Figure 2 illustrates that aPKC co-localizes with NFT. Using specific antisera to all known PKC gene products, immunohistochemistry reveals that only PKCζ and PKCι/λ are present in NFT. Silver stain confirms the presence of NFT in this tissue, and omitting primary antisera fails to label NFT.

Figure 3A illustrates a Western blot showing reactivity of aPKC antibodies in hippocampus, caudate nucleus, cerebellum, and superior temporal cortex. PKMζ was seen as a 55 kD band in all regions tested. PKCι/λ was seen as a 72 kD band in all regions tested. Full-length PKCζ was only seen in the cerebellum. Figure 3B illustrates the stability of aPKC in post-mortem rat brain tissue. The total level of aPKC was measured using quantitative Western blot with specific antisera to the catalytic domain of PKCζ and PKCι/λ.

Figure 4 illustrates that PKCγ and PKCι/λ but not PKMζ decreases in the superior temporal cortex in AD. Figure 4A: Representative Western blots on total protein homogenates from a representative control and AD case. Figure 4B: Histogram showing mean and standard deviation. * indicates statistical significance.

Figure 5 illustrates that PKCι/λ but not PKMζ or PKCγ increase in the caudate nucleus in AD. Figure 5A: Representative Western blots on total protein homogenates from a representative control and AD case. Figure 5B: Histogram showing mean and standard deviation. * indicates statistical significance.

Figure 6 illustrates localization of aPKC in human cerebral parahippocampal cortex. ζ-C1 (Figure 6B). No primary antibody control (Figure 6A). aPKC staining can be seen in perikarya and proximal dendrites. No neuronal nuclear staining was observed. Scale bar = 100 μm.

Figure 7 illustrates localization of aPKC in human astrocytes. Astrocytes stained strongly with antiPKCι/λ in the cerebral cortex (Figure 7A). ζ-C1 reacted with astrocytes in CA-4 of the hippocampus (Figure 7B). Scale bar =

200μm. Inset shows high power of an astrocyte. Scale bar = 25 μm.

Figure 8 illustrates localization of aPKC in ependymal glia. ζ-C1 reacted with ependymal glia (Figure 8B). No primary antibody control (Figure 8A). bar = 100 μm.

Figure 9 illustrates that aPKC colocalizes with neuropil threads and dystrophic neurites. Figure 9A: Immunohistochemical stain using z-C2 of paraffin embedded hippocampal cortex from case of AD. Figure 9B: Silver stain from a non-adjacent section. Large arrows indicate SP; small arrows indicate neuropil threads. bar = 100 μm.

Figure 10 illustrates that aPKC colocalizes with Hirano bodies (HB) in area CA-1 of the hippocampus. Arrows indicate HB can be seen as a brightly eosinophilic rod-like structure when stained with hematoxylin and eosin (H&E, Figure 10A). HB can be seen with all of the aPKC antibodies, ζ-C1 (Figure 10D), ζ-C2 (Figure 10B) and ι/λ (Figure 10C). bar = 100 μm.

Figure 11 illustrates PKMζ distribution correlates with unique brain-specific ζ RNA. Figure 11A: Reverse-transcription polymerase chain reaction (RT-PCR) using specific primers reveal that PKMζ mRNA is abundant only in brain; PKCζ mRNA is abundant in many other tissues. Figure 11B: RNase protection reveals levels of PKMζ mRNA and PKCζ mRNA in different tissues. Figure 11C: Northern blot to quantify levels of PKMζ mRNA and PKCζ mRNA in different tissues.

Figure 12 illustrates that treatment of dissociated primary hippocampal neuronal cultures with high potassium results in increased levels of PKMζ (green) reletive to MAP2 (red). Figure 12A: Control culture untreated has low levels of PKMζ. Figure 12B: Stimulation with high potassium results in an increase in PKMζ.

Figure 13 depicts a Western blot showing the lack of expression of PKCζI/II in neuroblastoma cells (IMR-32 cell line). Lane 1 shows that a rat brain lysate when probed with an anti-PKCi/l antiserum displays a band at about 72 kD. Lysates of IMR-32 cells (lane 2) and pheochromocytoma cells (lane 3), when probed with specific anti-PKCι/λ antisera also show PKCi/l. In contrast, when IMR32 and PC12 cells are probed with a specific anti-PKCζI/II antiserum, IMR32 cells have no

bands (lane 6), but PC12 cells show multiple bands (lane 7). When IMR-32 cells were transfected with PKCζII a doublet of two bands was detected for PKCζII at around 80 kDa (lane 5). In contrast, lysates of IMR-32 cells transfected with EGFP alone did not contain a band when probed with anti-PKCζII (lane 4).

Figure 14 depicts association of PKCι/λ with tauopathies and α-synucleinopathies. The PKCι/λ antibody weakly labeled cytoplasm of unremarkable hippocampal neurons and neuropil in a control case (Fig. 14a), while strongly labeling NFTs (Fig. 14b) and Hirano bodies (Fig. 14c) in an AD case. The antibody also labeled Pick bodies (Fig. 14d) and Pick cells in PiD; globose tangles (Fig. 14e) and tufted glial cells (Fig. 14f) in PSP; and ballooned neurons (Fig. 14g) and astrocytes (Fig. 14h) in CBD. In α-synucleinopathies, the antibody strongly labeled classic Lewy bodies in pigmented substantia nigra neurons (Fig. 14i) and Lewy bodies in amygdala (Fig. 14j) and cortex in PD and DLB. In MSA, glial inclusions were also immunoreactive (Fig. 14k). Omission of the primary antibody yielded no staining of any inclusions such as these nigral Lewy bodies (arrow) (Fig. 14l).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the atypical isoforms of protein kinase C (aPKC) and their participation in intracellular localization and mislocalization of cellular components, particularly in human diseases, e.g. Alzheimer's disease (AD) and cancer.

By "Activity" is meant the ability of an enzyme to act as a catalyst to induce chemical changes in other substances, including the ability to transfer phosphate to substrate molecules as well as to aggregate molecules. "Activity of aPKC" or "aPKC activity" used herein includes, for example, inhibition of aPKC, co-localization of aPKC with neurofibrillary tangles (NFT) or Hirano body (HB) and phosphorylation of protein tau by aPKC. "Activity" used herein can be also referred to the level, amount or localization of the enzyme.

By "control" is meant a normal subject or unchanged chemical, or a subject or a chemical in its normal state. "Control" can also be refered to as a chemical with known properties, for example, in the case of a "positive control." Thus, a "control" can be referred to as comparison to a reference range which is the normal value.

The PKC gene family consists of at least 10 distinct isoforms with diverse cellular functions (Newton, 2003). Although all of the PKC isoforms can theoretically have truncated PKM forms, the only PKM form that is consistently observed in the brain is PKMζ, an atypical member of the PKC family (aPKC) (Sacktor et al). Specifically, it has been discovered by the inventors that PKMζ, a key molecule in the maintenance of long-term potentiation (LTP) and memory formation in *Drosophila*, colocalizes with the NFT. Accordingly, disruption in PKMζ signaling may contribute to the pathogenesis of neurological disorders, such as AD.

By "disruption in PKMζ signaling" is meant increases or decreases in the level, location or activity of the kinase or its effectors, substrates and associated molecules.

"Modulating" or "modulate" used herein also include inhibiting or inhibit and activating or activate.

According to the present invention, alteration in aPKC function can lead to hyperphosphorylation of the cytoskeletal protein tau, see Figure 1. Also in accordance with the present invention, aPKC colocalizes with the NFT, see Figure 2.

Phosphorylation of tau at sites clustered around the MT binding regions inhibits tau binding to MT. Indeed, the tau found in AD brain and NFT is hyperphosphorylated at many sites not normally phosphorylated in adult human brain. The present invention recognizes that phosphorylation of tau causes its dissociation from MTs and buildup in the cytoplasm, which in turn could lead to the formation of tau aggregates and ultimately NFT.

In accordance with the present invention, the localization of aPKC to the NFT and the ability of aPKC to phosphorylate tau confirm that aPKC is altered in nervous system disorders, such as AD. Accordingly, co-localization of aPKC, especially PKMζ, with NFT strongly suggests aPKC is involved in tau phosphorylation. Accordingly, the present invention employs the activity of aPKC, especially PKMζ, and provides compositions and methods that are useful for diagnosis, drug screening and treatment of neurological disorders, such as AD.

"Psychological disorder" refers to a diseases of the mind in the context of human and animal behavior. "Neurological disorder" refers to diseases of

the nervous system. "Neurodegenrative disorder" refers to diseases characterized by progressive degeneration of neurons in the nervous system.

Accordingly, the present invention can be employed in detection, diagnosis, treatment, prevention or prognosis of nervous system disorders that include, but are not limited to, Acquired Epileptiform Aphasia, Acute Disseminated Encephalomyelitis, Adrenoleukodystrophy, Agenesis of the corpus callosum, Agnosia, Aicardi syndrome, Alexander disease, Alpers' disease, Alternating hemiplegia, Alzheimer's disease, Amyotrophic lateral sclerosis,Anencephaly, Angelman syndrome, Angiomatosis, Anoxia, Aphasia, Apraxia, Arachnoid Cysts, Arachnoiditis, Arnold-Chiari malformation, Arteriovenous malformation, Asperger syndrome, Ataxia Telangiectasia, Attention Deficit Hyperactivity Disorder, Autism, Autonomic Dysfunction, Back Pain, Batten disease, Behcet's disease, Bell's palsy, Benign Essential Blepharospasm, Benign Focal Amyotrophy, Benign Intracranial Hypertension, Binswanger's disease, Blepharospasm, Bloch-Sulzberger syndrome, Brachial plexus injury, Brain abscess, Brain injury, Brain tumor, Spinal tumor, Brown-Sequard syndrome, Canavan disease, Carpal tunnel syndrome,Causalgia, Central pain syndrome, Central pontine myelinolysis, Cephalic disorder, Cerebral aneurysm, Cerebral arteriosclerosis, Cerebral atrophy, Cerebral gigantism, Cerebral palsy, Charcot-Marie-Tooth disease, Chiari malformation, Chorea, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic pain, Chronic regional pain syndrome, Coffin Lowry syndrome, Coma, including Persistent Vegetative State, Congenital facial diplegia, Corticobasal degeneration, Cranial arteritis, Craniosynostosis, Creutzfeldt-Jakob disease, Cumulative trauma disorders, Cushing's syndrome, Cytomegalic inclusion body disease (CIBD), Cytomegalovirus Infection, Dancing eyes-dancing feet syndrome, Dandy-Walker syndrome, Dawson disease, De Morsier's syndrome, Dejerine-Klumpke palsy, Dementia, Dermatomyositis, Diabetic neuropathy, Diffuse sclerosis, Dysautonomia, Dysgraphia, Dyslexia, Dystonias, Early infantile epileptic encephalopathy, Empty sella syndrome, Encephalitis, Encephaloceles, Encephalotrigeminal angiomatosis, Epilepsy, Erb's palsy, Essential tremor, Fabry's disease, Fahr's syndrome, Fainting, Familial spastic paralysis, Febrile seizures, Fisher syndrome, Friedreich's ataxia, Gaucher's disease, Gerstmann's syndrome, Giant cell arteritis, Giant cell inclusion disease, Globoid cell Leukodystrophy, Guillain-Barre syndrome, HTLV-1 associated myelopathy,

Hallervorden-Spatz disease, Head injury, Headache, Hemifacial Spasm, Hereditary Spastic Paraplegia, Heredopathia atactica polyneuritiformis, Herpes zoster oticus, Herpes zoster, Hirayama syndrome, Holoprosencephaly, Huntington's disease, Hydranencephaly, Hydrocephalus, Hypercortisolism, Hypoxia, Immune-Mediated encephalomyelitis, Inclusion body myositis, Incontinentia pigmenti, Infantile phytanic acid storage disease, Infantile Refsum disease, Infantile spasms, Inflammatory myopathy, Intracranial cyst, Intracranial hypertension, Joubert syndrome, Kearns-Sayre syndrome, Kennedy disease, Kinsbourne syndrome, Klippel Feil syndrome, Krabbe disease, Kugelberg-Welander disease, Kuru, Lafora disease, Lambert-Eaton myasthenic syndrome, Landau-Kleffner syndrome, Lateral medullary (Wallenberg) syndrome, Learning disabilities, Leigh's disease, Lennox-Gastaut syndrome, Lesch-Nyhan syndrome, Leukodystrophy, Lewy body dementia, Lissencephaly, Locked-In syndrome, Lou Gehrig's disease,Lumbar disc disease, Lyme disease - Neurological Sequelae, Machado-Joseph disease, Macrencephaly, Megalencephaly, Melkersson-Rosenthal syndrome, Menieres disease, Meningitis, Menkes disease, Metachromatic leukodystrophy, Microcephaly, Migraine, Miller Fisher syndrome, Mini-Strokes, Mitochondrial Myopathies, Mobius syndrome, Monomelic amyotrophy, Motor Neurone Disease, Moyamoya disease, Mucopolysaccharidoses, Multi-Infarct Dementia, Multifocal motor neuropathy, Multiple sclerosis, Multiple system atrophy with postural hypotension, Muscular dystrophy, Myasthenia gravis, Myelinoclastic diffuse sclerosis, Myoclonic encephalopathy of infants, Myoclonus, Myopathy, Myotonia congenita, Narcolepsy, Neurofibromatosis, Neuroleptic malignant syndrome, Neurological manifestations of AIDS, Neurological sequelae of lupus, Neurological Sequelae of Lyme disease, Neuromyotonia, Neuronal ceroid lipofuscinosis, Neuronal migration disorders, Niemann-Pick disease, O'Sullivan-McLeod syndrome, Occipital Neuralgia, Occult Spinal Dysraphism Sequence, Ohtahara syndrome, Olivopontocerebellar Atrophy, Opsoclonus Myoclonus, Optic neuritis, Orthostatic Hypotension, Overuse syndrome, Pain - Chronic, Paresthesia, Parkinson's disease, Paramyotonia Congenita, Paraneoplastic diseases, Paroxysmal attacks, Parry Romberg syndrome, Pelizaeus-Merzbacher disease, Periodic Paralyses, Peripheral Neuropathy, Persistent Vegetative State, Pervasive Developmental Disorders, Photic sneeze reflex, Phytanic Acid Storage disease, Pick's disease, Pinched

Nerve, Pituitary Tumors, Polymyositis, Porencephaly, Post-Polio syndrome, Postherpetic Neuralgia, Postinfectious Encephalomyelitis, Postural Hypotension, Prader-Willi syndrome, Primary Lateral Sclerosis, Prion diseases, Progressive Hemifacial Atrophy, Progressive multifocal leukoencephalopathy, Progressive Sclerosing Poliodystrophy, Progressive Supranuclear Palsy, Pseudotumor cerebri, Ramsay-Hunt syndrome, Ramsay Hunt syndrome Type I, Ramsay Hunt syndrome Type II, Rasmussen's Encephalitis, Reflex Sympathetic Dystrophy syndrome, Refsum disease - Infantile, Refsum disease, Repetitive Motion Disorders, Repetitive Stress Injuries, Restless Legs syndrome, Retrovirus-Associated Myelopathy, Rett syndrome, Reye's syndrome, Saint Vitus Dance, Sandhoff disease, Schilder's disease, Schizencephaly, Septo-Optic Dysplasia, Shaken Baby syndrome, Shingles, Shy-Drager syndrome, Sjogren's syndrome, Sleep Apnea, Soto's syndrome, Spasticity, Spina bifida, Spinal Cord injury, Spinal Cord Tumors, Spinal Muscular Atrophy, Stiff-Person syndrome, Stroke, Sturge-Weber syndrome, Subacute Sclerosing Panencephalitis, Subcortical Arteriosclerotic Encephalopathy, Sydenham Chorea, Syncope, Syringomyelia, Tardive Dyskinesia, Tay-Sachs disease, Temporal arteritis, Tethered Spinal Cord syndrome, Thomsen disease, Thoracic Outlet syndrome, Tic Douloureux, Todd's Paralysis, Tourette syndrome, Transient ischemic attack, Transmissible Spongiform Encephalopathies, Transverse myelitis, Traumatic Brain injury, Tremor, Trigeminal Neuralgia, Tropical Spastic Paraparesis, Tuberous Sclerosis, Vasculitis including Temporal Arteritis, Von Hippel-Lindau Disease (VHL), Wallenberg's syndrome, Werdnig-Hoffman disease, West syndrome, Whiplash, Williams syndrome, Wilson's disease, Zellweger syndrome.

The present invention can also be employed in detection, diagnosis, treatment, prevention or prognosis of tauopathies (i.e., disorders involving protein tau based pathology), such as, Aging, Alzheimer's disease (familial, sporadic), Amyotrophic lateral sclerosis/Parkinsonism dementia complex of Guam, Argyrophilic grain disease, British type amyloid angiopathy, Corticobasal degeneration, Dementia pugilistica/autism with self-injury behaviour, Down's syndrome, Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17), Gerstmann-Straussler-Scheinker disease, Hallenvorden-Spatz disease, Inclusion body myositis, Multisystem atophy, Myotonic dystrophy, Niemann-Pick

disease type C, Parkinson with dementia of Guadeloupe, Pick's disease, Presenile dementia with tangles and calcifications, Prion protein cerebral amyloid angiopathy, Progressive supranuclear palsy, Post-encephalitic parkinsonism, Subacute sclerosing panencephalitis, Tangle only dementia.

It is also encompassed by the present invention for detection, diagnosis, treatment, prevention or prognosis of psychiatric disorders including Academic Problem, Acculturation Problem, Acute Stress Disorder, Adjustment Disorder Unspecified, Adjustment Disorder With Anxiety, Adjustment Disorder With Depressed Mood, Adjustment Disorder With Disturbance of Conduct, Adjustment Disorder With Mixed Anxiety and Depressed Mood, Adjustment Disorder With Mixed Disturbance of Emotions and Conduct, Adult Antisocial Behavior, Adverse Effects of Medication NOS, Age-Related Cognitive Decline, Agoraphobia Without History of Panic Disorder, Alcohol Abuse, Alcohol Dependence, Alcohol Intoxication, Alcohol Intoxication Delirium, Alcohol Withdrawal, Alcohol Withdrawal Delirium, Alcohol-Induced Anxiety Disorder, Alcohol-Induced Mood Disorder, Alcohol-Induced Persisting Amnestic Disorder, Alcohol-Induced Persisting Dementia, Alcohol-Induced Psychotic Disorder, With Delusions, Alcohol-Induced Psychotic Disorder, With Hallucinations, Alcohol-Induced Sexual Dysfunction, Alcohol-Induced Sleep Disorder, Alcohol-Related Disorder NOS, Amnestic Disorder, Amnestic Disorder NOS, Amphetamine Abuse, Amphetamine Dependence, Amphetamine Intoxication, Amphetamine Intoxication Delirium, Amphetamine Withdrawal, Amphetamine-Induced Anxiety Disorder, Amphetamine-Induced Mood Disorder, Amphetamine-Induced Psychotic Disorder, With Delusions, Amphetamine-Induced Psychotic Disorder, With Hallucinations, Amphetamine-Induced Sexual Dysfunction, Amphetamine-Induced Sleep Disorder, Amphetamine-Related Disorder NOS, Anorexia Nervosa, Antisocial Personality Disorder, Anxiety Disorder, Anxiety Disorder NOS, Asperger's Disorder, Attention-Deficit/Hyperactivity Disorder NOS, Attention-Deficit/Hyperactivity Disorder, Combined Type, Attention-Deficit/Hyperactivity Disorder, Predominantly Hyperactive-Impulsive Type, Attention-Deficit/Hyperactivity Disorder, Predominantly Inattentive Type, Autistic Disorder, Avoidant Personality Disorder, Bereavement, Bipolar Disorder NOS, Bipolar I Disorder, Most Recent Episode Depressed, In Full Remission, Bipolar I Disorder,

Most Recent Episode Depressed, In Partial Remission, Bipolar I Disorder, Most Recent Episode Depressed, Mild, Bipolar I Disorder, Most Recent Episode Depressed, Moderate, Bipolar I Disorder, Most Recent Episode Depressed, Severe With Psychotic Features, Bipolar I Disorder, Most Recent Episode Depressed, Severe Without Psychotic Features, Bipolar I Disorder, Most Recent Episode Depressed, Unspecified, Bipolar I Disorder, Most Recent Episode Hypomanic, Bipolar I Disorder, Most Recent Episode Manic, In Full Remission, Bipolar I Disorder, Most Recent Episode Manic, In Partial Remission, Bipolar I Disorder, Most Recent Episode Manic, Mild, Bipolar I Disorder, Most Recent Episode Manic, Moderate, Bipolar I Disorder, Most Recent Episode Manic, Severe With Psychotic Features, Bipolar I Disorder, Most Recent Episode Manic, Severe Without Psychotic Features, Bipolar I Disorder, Most Recent Episode Manic, Unspecified, Bipolar I Disorder, Most Recent Episode Mixed, In Full Remission, Bipolar I Disorder, Most Recent Episode Mixed, In Partial Remission, Bipolar I Disorder, Most Recent Episode Mixed, Mild, Bipolar I Disorder, Most Recent Episode Mixed, Moderate, Bipolar I Disorder, Most Recent Episode Mixed, Severe With Psychotic Features, Bipolar I Disorder, Most Recent Episode Mixed, Severe Without Psychotic Features, Bipolar I Disorder, Most Recent Episode Mixed, Unspecified, Bipolar I Disorder, Most Recent Episode Unspecified, Bipolar I Disorder, Single Manic Episode, In Full Remission, Bipolar I Disorder, Single Manic Episode, In Partial Remission, Bipolar I Disorder, Single Manic Episode, Mild, Bipolar I Disorder, Single Manic Episode, Moderate, Bipolar I Disorder, Single Manic Episode, Severe With Psychotic Features, Bipolar I Disorder, Single Manic Episode, Severe Without Psychotic Features, Bipolar I Disorder, Single Manic Episode, Unspecified, Bipolar II Disorder, Body Dysmorphic Disorder, Borderline Intellectual Functioning, Borderline Personality Disorder, Breathing-Related Sleep Disorder, Brief Psychotic Disorder, Bulimia Nervosa, Caffeine Intoxication, Caffeine-Induced Anxiety Disorder, Caffeine-Induced Sleep Disorder, Caffeine-Related Disorder NOS, Cannabis Abuse, Cannabis Dependence, Cannabis Intoxication, Cannabis Intoxication Delirium, Cannabis-Induced Anxiety Disorder, Cannabis-Induced Psychotic Disorder, With Delusions, Cannabis-Induced Psychotic Disorder, With Hallucinations, Cannabis-Related Disorder NOS,

Catatonic Disorder, Child or Adolescent Antisocial Behavior, Childhood Disintegrative Disorder, Chronic Motor or Vocal Tic Disorder, Circadian Rhythm Sleep Disorder, Cocaine Abuse, Cocaine Dependence, Cocaine Intoxication, Cocaine Intoxication Delirium, Cocaine Withdrawal, Cocaine-Induced Anxiety Disorder, Cocaine-Induced Mood Disorder, Cocaine-Induced Psychotic Disorder, With Delusions, Cocaine-Induced Psychotic Disorder, With Hallucinations, Cocaine-Induced Sexual Dysfunction, Cocaine-Induced Sleep Disorder, Cocaine-Related Disorder NOS, Cognitive Disorder NOS, Communication Disorder NOS, Conduct Disorder, Conversion Disorder, Cyclothymic Disorder, Delirium Due to[Indicate the General Medical Condition], Delirium NOS, Delusional Disorder, Dementia Due to Creutzfeldt-Jakob Disease, Dementia Due to Head Trauma, Dementia Due to HIV Disease, Dementia Due to Huntington's Disease, Dementia Due to Parkinson's Disease, Dementia Due to Pick's Disease, Dementia Due to[Indicate the General Medical Condition], Dementia NOS, Dementia of the Alzheimer's Type, With Early Onset, Uncomplicated, Dementia of the Alzheimer's Type, With Early Onset, With Delirium, Dementia of the Alzheimer's Type, With Early Onset, With Delusions, Dementia of the Alzheimer's Type, With Early Onset, With Depressed Mood, Dementia of the Alzheimer's Type, With Late Onset, Uncomplicated, Dementia of the Alzheimer's Type, With Late Onset, With Delirium, Dementia of the Alzheimer's Type, With Late Onset, With Delusions, Dementia of the Alzheimer's Type, With Late Onset, With Depressed Mood, Dependent Personality Disorder, Depersonalization Disorder, Depressive Disorder NOS, Developmental Coordination Disorder, Diagnosis Deferred on Axis II, Diagnosis or Condition Deferred on Axis I, Disorder of Infancy, Childhood, or Adolescence NOS, Disorder of Written Expression, Disruptive Behavior Disorder NOS, Dissociative Amnesia, Dissociative Disorder NOS, Dissociative Fugue, Dissociative Identity Disorder, Dyspareunia (Not Due to a General Medical Condition), Dyssomnia NOS, Dysthymic Disorder, Eating Disorder NOS, Encopresis, With Constipation and Overflow Incontinence, Encopresis, Without Constipation and Overflow Incontinence, Enuresis (Not Due to a General Medical Condition), Exhibitionism, Expressive Language Disorder, Factitious Disorder NOS, Factitious Disorder With Combined Psychological and Physical Signs and Symptoms, Factitious Disorder

With Predominantly Physical Signs and Symptoms, Factitious Disorder With Predominantly Psychological Signs and Symptoms, Feeding Disorder of Infancy or Early Childhood, Female Dyspareunia, Female Hypoactive Sexual Desire Disorder, Female Orgasmic Disorder, Female Sexual Arousal Disorder, Fetishism, Frotteurism, Gender Identity Disorder in Adolescents or Adults, Gender Identity Disorder in Children, Gender Identity Disorder NOS, Generalized Anxiety Disorder, Hallucinogen Abuse, Hallucinogen Dependence, Hallucinogen Intoxication, Hallucinogen Intoxication Delirium, Hallucinogen Persisting Perception Disorder, Hallucinogen-Induced Anxiety Disorder, Hallucinogen-Induced Mood Disorder, Hallucinogen-Induced Psychotic Disorder, With Delusions, Hallucinogen-Induced Psychotic Disorder, With Hallucinations, Hallucinogen-Related Disorder NOS, Histrionic Personality Disorder, Hypersomnia, Hypoactive Sexual Desire Disorder, Hypochondriasis, Identity Problem, Impulse-Control Disorder NOS, Inhalant Abuse, Inhalant Dependence, Inhalant Intoxication, Inhalant Intoxication Delirium, Inhalant-Induced Anxiety Disorder, Inhalant-Induced Mood Disorder, Inhalant-Induced Persisting Dementia, Inhalant-Induced Psychotic Disorder, With Delusions, Inhalant-Induced Psychotic Disorder, With Hallucinations, Inhalant-Related Disorder NOS, Insomnia, Intermittent Explosive Disorder, Kleptomania, Learning Disorder NOS, Major Depressive Disorder, Recurrent, In Full Remission, Major Depressive Disorder, Recurrent, In Partial Remission, Major Depressive Disorder, Recurrent, Mild, Major Depressive Disorder, Recurrent, Moderate, Major Depressive Disorder, Recurrent, Severe With Psychotic Features, Major Depressive Disorder, Recurrent, Severe Without Psychotic Features, Major Depressive Disorder, Recurrent, Unspecified, Major Depressive Disorder, Single Episode, In Full Remission, Major Depressive Disorder, Single Episode, In Partial Remission, Major Depressive Disorder, Single Episode, Mild, Major Depressive Disorder, Single Episode, Moderate, Major Depressive Disorder, Single Episode, Severe With Psychotic Features, Major Depressive Disorder, Single Episode, Severe Without Psychotic Features, Major Depressive Disorder, Single Episode, Unspecified, Male Dyspareunia, Male Erectile Disorder, Male Hypoactive Sexual Desire Disorder, Male Orgasmic Disorder, Malingering, Mathematics Disorder, Medication-Induced Movement Disorder NOS, Medication-Induced Postural

Tremor, Mental Disorder NOS, Mental Retardation, Severity Unspecified, Mild Mental Retardation, Mixed Receptive-Expressive Language Disorder, Moderate Mental Retardation, Mood Disorder, Mood Disorder NOS, Narcissistic Personality Disorder, Narcolepsy, Neglect of Child, Neglect of Child (if focus of attention is on victim), Neuroleptic Malignant Syndrome, Neuroleptic-Induced Acute Akathisia, Neuroleptic-Induced Acute Dystonia, Neuroleptic-Induced Parkinsonism, Neuroleptic-Induced Tardive Dyskinesia, Nicotine Dependence, Nicotine Withdrawal, Nicotine-Related Disorder NOS, Nightmare Disorder, No Diagnosis on Axis II, No Diagnosis or Condition on Axis I, Noncompliance With Treatment, Obsessive-Compulsive Disorder, Obsessive-Compulsive Personality Disorder, Occupational Problem, Opioid Abuse, Opioid Dependence, Opioid Intoxication, Opioid Intoxication Delirium, Opioid Withdrawal, Opioid-Induced Mood Disorder, Opioid-Induced Psychotic Disorder, With Delusions, Opioid-Induced Psychotic Disorder, With Hallucinations, Opioid-Induced Sexual Dysfunction, Opioid-Induced Sleep Disorder, Opioid-Related Disorder NOS, Oppositional Defiant Disorder, Other (or Unknown) Substance Abuse, Other (or Unknown) Substance Dependence, Other (or Unknown) Substance Intoxication, Other (or Unknown) Substance Withdrawal, Other (or Unknown) Substance-Induced Anxiety Disorder, Other (or Unknown) Substance-Induced Delirium, Other (or Unknown) Substance-Induced Mood Disorder, Other (or Unknown) Substance-Induced Persisting Amnestic Disorder, Other (or Unknown) Substance-Induced Persisting Dementia, Other (or Unknown) Substance-Induced Psychotic Disorder, With Delusions, Other (or Unknown) Substance-Induced Psychotic Disorder, With Hallucinations, Other (or Unknown) Substance-Induced Sexual Dysfunction, Other (or Unknown) Substance-Induced Sleep Disorder, Other (or Unknown) Substance-Related Disorder NOS, Other Female Sexual Dysfunction, Other Male Sexual Dysfunction, Pain Disorder Associated With Both Psychological Factors and a General Medical Condition, Pain Disorder Associated With Psychological Factors, Panic Disorder With Agoraphobia, Panic Disorder Without Agoraphobia, Paranoid Personality Disorder, Paraphilia NOS, Parasomnia NOS, Parent-Child Relational Problem, Partner Relational Problem, Pathological Gambling, Pedophilia, Personality Change, Personality Disorder NOS, Pervasive Developmental Disorder NOS, Phase of Life Problem, Phencyclidine Abuse,

Phencyclidine Dependence, Phencyclidine Intoxication, Phencyclidine Intoxication Delirium, Phencyclidine-Induced Anxiety Disorder, Phencyclidine-Induced Mood Disorder, Phencyclidine-Induced Psychotic Disorder, With Delusions, Phencyclidine-Induced Psychotic Disorder, With Hallucinations, Phencyclidine-Related Disorder NOS, Phonological Disorder, Physical Abuse of Adult, Physical Abuse of Adult (if focus of attention is on victim), Physical Abuse of Child, Physical Abuse of Child (if focus of attention is on victim), Pica, Polysubstance Dependence, Posttraumatic Stress Disorder, Premature Ejaculation, Primary Hypersomnia, Primary Insomnia, Profound Mental Retardation, Psychotic Disorder, With Delusions, Psychotic Disorder, With Hallucinations, Psychotic Disorder NOS, Pyromania, Reactive Attachment Disorder of Infancy or Early Childhood, Reading Disorder, Relational Problem NOS, Relational Problem Related to a Mental Disorder or General Medical Condition, Religious or Spiritual Problem, Rett's Disorder, Rumination Disorder, Schizoaffective Disorder, Schizoid Personality Disorder, Schizophrenia, Catatonic Type, Schizophrenia, Disorganized Type, Schizophrenia, Paranoid Type, Schizophrenia, Residual Type, Schizophrenia, Undifferentiated Type, Schizophreniform Disorder, Schizotypal Personality Disorder, Sedative, Hypnotic, or Anxiolytic Abuse, Sedative, Hypnotic, or Anxiolytic Dependence, Sedative, Hypnotic, or Anxiolytic Intoxication, Sedative, Hypnotic, or Anxiolytic Intoxication Delirium, Sedative, Hypnotic, or Anxiolytic Withdrawal, Sedative, Hypnotic, or Anxiolytic Withdrawal Delirium, Sedative-, Hypnotic-, or Anxiolytic-Induced Anxiety Disorder, Sedative-, Hypnotic-, or Anxiolytic-Induced Mood Disorder, Sedative-, Hypnotic-, or Anxiolytic-Induced Persisting Amnestic Disorder, Sedative-, Hypnotic-, or Anxiolytic-Induced Persisting Dementia, Sedative-, Hypnotic-, or Anxiolytic-Induced Psychotic Disorder, With Delusions, Sedative-, Hypnotic-, or Anxiolytic-Induced Psychotic Disorder, With Hallucinations, Sedative-, Hypnotic-, or Anxiolytic-Induced Sexual Dysfunction, Sedative-, Hypnotic-, or Anxiolytic-Induced Sleep Disorder, Sedative-, Hypnotic-, or Anxiolytic-Related Disorder NOS, Selective Mutism, Separation Anxiety Disorder, Severe Mental Retardation, Sexual Abuse of Adult, Sexual Abuse of Adult (if focus of attention is on victim), Sexual Abuse of Child, Sexual Abuse of Child (if focus of attention is on victim), Sexual Aversion Disorder, Sexual Disorder NOS,

Sexual Dysfunction NOS, Sexual Masochism, Sexual Sadism, Shared Psychotic Disorder, Sibling Relational Problem, Sleep Disorder, Hypersomnia Type, Insomnia Type, Mixed Type, Parasomnia Type, Sleep Terror Disorder, Sleepwalking Disorder, Social Phobia, Somatization Disorder, Somatoform Disorder NOS, Specific Phobia, Stereotypic Movement Disorder, Stuttering, Tic Disorder NOS, Tourette's Disorder, Transient Tic Disorder, Transvestic Fetishism, Trichotillomania, Undifferentiated Somatoform Disorder, Unspecified Mental Disorder (nonpsychotic), Vaginismus (Not Due to a General Medical Condition), Vascular Dementia, Uncomplicated, Vascular Dementia, With Delirium, Vascular Dementia, With Delusions, Vascular Dementia, With Depressed Mood, Voyeurism.

"Label," "labeled" or "detectable labeled" refers to incorporation of a detectable marker, for example by incorporation of a radioactively labeled compound or attachment to a polypeptide of moieties such as biotin that can be detected by the binding of a section moiety, such as marked avidin. Various methods of labeling polypeptide, nucleic acids, carbohydrates, and other biological or organic molecules are known in the art. Such labels can have a variety of readouts, such as radioactivity, fluorescence, color, chemiluminescence or other readouts known in the art or later developed. The readouts can be based on enzymatic activity, such as beta-galactosidase, beta-lactamase, horseradish peroxidase, alkaline phosphatase, luciferase; radioisotopes such as ^{3}H, ^{14}C, ^{35}S, ^{125}I or ^{131}I); fluorescent proteins, such as green fluorescent proteins (GFP); or other fluorescent labels, such as FITC, rhodamine, and lanthanides. Where appropriate, these labels can be the product of the expression of reporter genes, as that term is understood in the art. Examples of reporter genes are beta-lactamase (U.S. Pat. No. 5,741,657 to Tsien et al., issued April 21, 1998) and green fluorescent protein (U.S. Pat. No. 5,777,079 to Tsien et al., issued July 7, 1998; U.S. Pat. No. 5,804,387 to Cormack et al., issued September 8, 1998).

A "test chemical" or "test compound" or "candidate compound" refers to a chemical, composition or extract to be tested by at least one method of the present invention to be a putative modulator. A test chemical can be of any chemical composition, such as inorganic, organic or a biomolecule. A biomolecule can be any molecule of biological origin that is found in or produced at least in part

by a cell, and include, but are not limited to polypeptides, nucleic acids, lipids, carbohydrates or combinations thereof. A test chemical is usually not known to bind to the target of interest. "Indentifying a compound" or "screening a drug" used herein refers to a process to determine the putative modulating or therapeutic function of a candidate compound.

"Control test chemical" or "control compound" refers to a chemical known to bind to the target (for example, a known agonist, antagonist, partial agonist or inverse agonist). Test chemical does not typically include a chemical added to a mixture as a control condition that alters the function of the target to determine signal specificity in an assay. Such control chemicals or conditions include chemicals that (1) non-specifically or substantially disrupt protein structure (for example denaturing agents such as urea or guandium, sulfhydryl reagents such as dithiotritol and beta-mercaptoethanol), (2) generally inhibit cell metabolism (for example mitochondrial uncouples) and (3) non-specifically disrupt electrostatic or hydrophobic interactions of a protein (for example, high salt concentrations or detergents at concentrations sufficient to non-specifically disrupt hydrophobic or electrostatic interactions). The term "test chemical" or "candidate compound" also does not typically include chemicals known to be unsuitable for a therapeutic use for a particular indication due to toxicity of the subject. Usually, various predetermined concentrations of test chemicals are used for determining their activity. If the molecular weight of a test chemical is known, the following ranges of concentrations can be used: between about 0.001 micromolar and about 10 millimolar, preferably between about 0.01 micromolar and about 1 millimolar, more preferably between about 0.1 micromolar and about 100 micromolar. When extracts are uses a test chemicals, the concentration of test chemical used can be expressed on a weight to volume basis. Under these circumstances, the following ranges of concentrations can be used: between about 0.001 micrograms/ml and about 1 milligram/ml, preferably between about 0.01 micrograms/ml and about 100 micrograms/ml, and more preferably between about 0.1 micrograms/ml and about 10 micrograms/ml.

"Macromolecule" refers to a molecule with a molecular mass greater than a one thousand daltons, such as a protein, nucleic acid, or polysaccharide.

"Treating" or "treatment" as used herein means to ameliorate,

suppress, mitigate or eliminate the clinical symptoms after the onset (i.e., clinical manifestation) of a disease state, such as an AD, including, but not limited to inhibiting neuronal degeneration or neuronal death, promoting or stimulating neuronal growth such that the symptoms of the disease condition are prevented or alleviated. Such treatment can include chemicals, such as chemotherapeutic agents or test compounds and/or non-chemical treatment, such as electrical pules (such as electroinnovation), magnetic fields or radiation (such as radiation therapy) (see, for example, Buonanno et al., Nucleic Acids Res. 20:539-544 (1992)). An effective or successful treatment provides a clinically observable improvement.

By "candidate treatment process" is meant administration of a candidate compound to a subject, or modification of environment, diet, behavior of the subject. By "control treatment process" is meant that the nature status or state of a subject being maintained without any treating or modification, or a treatment process having known references or properties is performed.

By "prevent", "preventing" or "prevention' is meant that chances of regaining and/or worsening and/or progression of the symptoms of a neurological diseases, such as, for example, AD, are lowered, reduced or eliminated after the symptoms are ameliorated, suppressed, mitigated or eliminated by the treatment.

A "specific binding member" refers to a member of a group of two or more moieties that can specifically bind with each other rather than becoming non-specifically associated with each other, such as by precipitation. Examples of specific binding members include, but are not limited to, antigen-antibody, receptor-ligand and nucleic acid-nucleic acid pairs.

"Specific," "specifically," "specifically bind" or a "specific binding" in the context of the binding of first specific binding member with at least one other specific binding member refers to binding that is preferential and not non-specific. Preferably, a specific binding reaction is unique for the specific binding members, but that need not be the case.

"Detectably bind" or "detectable binding" refers to the specific binding of one specific binding member with at least one other specific binding member that can be detected. For example, one specific binding member can be detectably labeled such that the detectable presence of the label indicates a specific

binding event. The detection limits of such detectable binding are related to the detectable label used and the detection method or device used.

An "antibody" refers to an immunoglobulin of any class or subclass, a portion thereof or an active fragment thereof, wherein an active fragment of an antibody retains its specific binding capability. An antibody can be a polyclonal antibody, a monoclonal antibody or a mixture thereof.

By "probe" is meant a substance, preferably a biomolecule, such as a fragment of DNA sequence or an antibody, which is labeled or otherwise marked and used to detect or identify another substance in a sample. An example of a probe can be a biomolecule that is labeled with radioactive isotopes or with a fluorescent marker that selectively binds to a specific gene so it can be isolated or identified or a strand of nucleic acid which can be labeled and used to hybridize to a complementary molecule from a mixture of other nucleic acids. The term "probe" used herein can be also referred to a single-stranded nucleic acid molecule with a known nucleotide sequence which is labeled in some way (for example, radioactively, fluorescently, or immunologically) and used to find and mark certain DNA or RNA sequences of interest to a researcher by hybridizing to it. A specific example of the probe used in the present invention is anti-aPKC antiserum.

A "specific probe" such as used in "aPKC specifc probe" is refered to any probe that specifically binds to aPKC gene or gene product(s). Preferably, such specific binding is a detectable binding.

A "tissue" refers to a collection of cells as known in the art. A "culture" of cells is a collection of cells as known in the art and can be a clonal population of cells or a mixed population of cells. A "tumor tissue" is a collection of cells that includes at least one cell derived from at least one tumor.

A "tissue extract" refers to a preparation that is derived from at least one source of tissue that has been treated such that the tissue and/or at least one cellular component of the cells in the tissue is no longer in its natural state or environment. For example, a tissue extract can be made by rupturing tissue using methods known in the art.

A "sample" includes any physical sample that includes a cell or a cell extract from a cell, a tissue, a biopsy sample, a tissue extract, for example. A sample

can be from a biological source such as a subject or animal or a portion thereof, or from a cell culture. Samples from a biological source can be from a normal or abnormal organism (such as an organism suffering from a condition or disease state, such as a neoplasm) or portion thereof and can be from any fluid, tissue or organ, including healthy or abnormal (such as diseased or neoplastic) fluids, tissues or organs. Samples from a subject or animal can be used in the present invention as obtained from the subject or animal, processed such as by sectioning, aspiration such as for bone marrow specimens or cultured such that cells from the sample can be sustained in vitro as a primary or continuous cell culture or cell line. For example, a sample in the present invention can be a tissue biopsy, cerebrospinal fluid (CSF), or a body fluid, such as blood, sample. In particular, a "tumor sample" is a sample that includes at least one cell derived from at least one tumor.

"Diagnosing" or "diagnosis" refers to the determination of whether a subject comprises a disease or condition, such as a nervous system disorder or cancer. "Diagnosing" also refers to distinguishing one cancer from another.

By "prognosing" or "prognosis" is meant the determination or prediction of the course of a disease or condition, such as cancer. The course of a disease or condition can be prognosed, for example, based on life expectancy or quality of life. Prognosing includes the determination of the time course of a disease or condition, with or without a treatment or treatments. In the instance where treatment(s) are contemplated, the prognosing includes prognosing the efficacy of a treatment for a disease or condition, such as cancer, or prognosing a malignancy.

A "control sample" refers to a sample that acts as a positive or negative control as they are known in the art and as appropriate for a particular assay. A control can be performed contemporaneously with an assay or be performed at a prior or later time. The results of an assay can be compared to a control to determine the validity of the assay. Controls can also be used to produce standard curves such that the results of an assay can be semi-quantitative or quantitative in nature. A reference range, i.e., a known range of normal values, can also be used as a control.

By "reference DNA" or "reference DNA sequence" is meant a DNA sequence, where its sequence is known and normal, i.e., without any mutation, and

where it encompasses the same region in the genome of a species as a DNA molecule to be compared, assayed or analyzed.

By "promoter" or "promoter region" is meant a nucleic acid sequence that controls expression of a coding sequence or a gene of interest. By "control expresion" is meant controlling production of RNA (e.g., mRNA or non-coding transcripts) by providing the recognition site for RNA polymerase and/or other factors necessary for starting transcription at the correct site. A promoter or promoter region usually resides upstream (5') of a coding sequence. As contemplated by the present invention, a promoter or promoter region can include variations of promoters derived by means of ligation to various regulatory sequences, random or controlled mutagenesis, and addition or duplication of enhancer sequences.

By "transgene" is meant any nucleic acid sequence non-native to a cell or an organism into which the nucleic acid sequence is transformed. "Transgene" also encompasses the component parts of a native gene of an organism modified by insertion of a non-native nucleic acid sequence by directed recombination.

The present invention is directed to the identification of the amount, localization and activity of atypical isoforms of protein kinase C (aPKC), e.g. PKMζ, and their role in disorders of the nervous system, such as Alzheimer's disease (AD).

Accordingly, one embodiment of the present invention is directed to diagnosis of AD. According to the present invention, a method for diagnosing neurological dysfunctions or nervous system disorders in a subject, using an aPKC specific probe, is provided. The diagnostic method of the present invention includes the steps of:

a. contacting a sample from the subject, e.g. a tissue biopsy, cerebrospinal fluid (CSF), or a blood sample, with an aPKC specific probe,
b. detecting the binding of the probe to the sample to determine the amount, localization or activity of the aPKC in the sample,
c. contacting a control sample with the aPKC specific probe,

d. detecting the binding of the probe to the control sample, e.g. a tissue biopsy, CSF, or a blood sample, to determine the amount, localization or activity of the aPKC in the control sample, and

e. comparing the amount, localization or activity of the aPKC in Step b with the amount, localization or activity of the aPKC in Step d, whereby if the amount, localization or activity of the aPKC in Step b is different from the amount, localization or activity of the aPKC in Step d, a nervous system disorder in the subject is present.

In accordance with the present invention, using probes to aPKC isoforms in the form of antibodies or nucleic acid, one can detect changes of aPKC in tissues from a subject through conventional techniques (Maniatis et al., *J. Molecular Cloning*, a laboratory manual, 1987; Ausubel, F.M., *Current Protocols in Molecular Biology*, 1987), and thereby ascertain whether an individual has a neurological disease, such as AD, or is likely to develop one. These probes can be used on postmortem autopsy tissue, brain biopsy tissue or any other tissue, tissue extract or body fluid (e.g., blood). In accordance with the present invention, a preferred probe is the antisense probe described in Example 11. Also in accordance with the present invention, using gene analysis for mutations in the aPKCs, one can detect changes of aPKC in tissues from a subject through conventional techniques (Ausubel, F.M., *Current Protocols in Molecular Biology*, 1987), and thereby ascertain whether an individual has a neurological disease or is likely to develop one.

Thus, it is an object of the present invention to detect neurological disorders, such as AD, at an early stage in order to treat and/or prevent the disorder.

Another embodiment of the present invention is directed to a method for screening drugs or molecular compounds useful for modulating the amount or activity of the aPKC. According to the present invention, a method for screening molecular compounds, e.g. peptides and small molecules, using cells and aPKC specific probes is provided. Such method includes:

a. providing cells wherein aPKC gene is expressed,

b. incubating the cells with a candidate compound for a sufficient time to induce a change in the amount, localization or activity of

aPKC in the cells,

c. incubating the cells as in Step b, in the absence of the candidate compound,

d. contacting the cells from Step b and Step c with an equal amount of an aPKC-specific probe,

e. detecting the binding of the probe to the cells to determine the amount, localization or activity of the aPKC in the cell from Step b and Step c, and

f. comparing the amount, localization or activity of the aPKC in the cells, wherein if the amount, localization or activity of the aPKC in the cells from Step b is different from the amount, localization or activity of the aPKC in the cells from Step c, the candidate compound is identified as a compound that modulates aPKC.

An alternate method for screening molecular compounds for use in treatment of brain disorders involves the over-expression or deletion of genetic elements containing atypical PKC sequences in cells or animals, e.g. mice, and assaying said cells or animals for atypical PKC activity or levels.

In accordance with present invention, the aPKC isoforms are used as rational targets for drug design for the purpose of treating and/or preventing AD and other neurological disorders. There are numerous methods currently available for testing the ability of drugs to influence the activity of proteins (Enna, S. J., Current Protocols in Pharmacology, 1998). The present invention is directed to the discovery that aPKC plays a key role in neurological disease and that changes in aPKC can be used to screen for useful drugs.

According to the present invention, a preferred compound is chelerythrine chloride (a PKC inhibitor), the zeta-inhibitory peptide (ZIP), or a selective aPKC inhibitor. An example of ZIP is myristoylated PKCζ pseudosubstrate peptide, i.e., Myr-SIYRRGARRWRKLY (SEQ ID NO: 10). See, Standaert et al., 1999, J. Biol. Chem. 274(20):14074-78.

According to the present invention, drugs that modulate aPKC can be screened by employing methodologies and related reagents that are known to the persons skilled in the art. Three such conventional methodologies have been developed, in both *in vivo* and *in vitro* systems. The first is a kinase assay using

recombinant expressed PKMζ in a baculovirus system. The second is a cell culture system using immunofluorescence. The third is using recombinant aPKC transfected into cultured cells. See, Ausubel, F.M., *Current Protocols in Molecular Biology*, 1987.

In accordance with the present invention, an aPKC, including PKMζ, contains a non-conventional ATP binding site motif GXGXXA (SEQ ID NO: 8), wherein G is amino acid glycine, X is any amino acid. This special characteristic of aPKC makes it less likely that drugs targeting these molecules will have harmful side effects comparing to other kinases or other PKC isoforms, which have ATP binding site hallmark motif GXGXXG (SEQ ID NO: 9).

In a particular embodiment of the present invention, an *in vitro* cell-free method for screening drugs or molecular compounds useful for modulating the amount, localization or activity of the aPKC, using aPKC and a substrate, is provided. Such method includes:

a. incubating an aPKC in a first container with adenosine triphosphate (ATP), preferably radioactively labeled ATP, a substrate of the aPKC, e.g. protein tau, with a candidate compound, for a sufficient time to induce a change in the amount or activity of the aPKC in said first containner,
b. incubating the aPKC in a second container with ATP, preferably radioactively labeled ATP, and the substrate of said aPKC, e.g. protein tau, for a sufficient time to induce a change in the amount or activity of aPKC in said second container,
c. quantifying the amount of incorporation of the phosphate into the substrate in the first container and the second container, and
d. comparing the amount of incorporation in the first container and the second container, whereby a difference in the amount of incorporation in the first container and the second container is indicative that the candidate compound is identified as a compound modulating aPKC amount, localization or activity.

A variation of this method involves using a phospho-specific antisera to quantify phosphate incorporation into the substrate.

Still another embodiment of the present invention is directed to a method, including gene therapy, for treating and/or preventing neurological disorders. According to the present invention, a method for treating and/or preventing neurological dysfunction is provided which employs a sequence corresponding to an aPKC, e.g. PKMζ (or an antisense sequence of aPKC) and an expression vector. This method includes the steps of:

a. inserting the aPKC sequence, e.g. PKMζ, into the expression vector, and

b. administering the vector to a subject, e.g. a patient,

whereby the vector treats and/or prevents a neurological dysfunction.

The methods of the present invention can be employed in the treatment and/or prevention of a variety of neurological disorders characterized by abnormal aPKC activity, such as phosphorylation of protein tau and co-localization with NFT. Such tau-associated filamentous aggregates are neuropathological hallmarks of Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD), Parkinson's Disease (PD), for example.

According to the present invention, aPKC function is altered in AD and other neurological disorders. Thus, in accordance with the present invention, aPKC and mutated forms, e.g. inactivated form of kinase, can be introduced to treat and/or prevent AD and other neurological disorders. Using gene therapy, the aPKC cDNA, preferably PKMζ cDNA, sequences or the mutated aPKC sequences, can be introduced to treat these diseases.

Such sequences are preferably provided in an expression vector. Expression vectors for use in the present methods include any appropriate gene therapy vectors, such as nonviral (e.g., plasmid vectors), retroviral, adenoviral, herpes simplex viral, adeno-associated viral, polio viruses and vaccinia vectors. Examples of retroviral vectors include, but are not limited to, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV)-derived recombinant vectors. Multiple teachings of gene therapy are available to those skilled in the art (Anderson, W. F., *Science*, 288: 627-629, 2000l; Anderson, W. F., *Science*, 226: 401-409, 1984; Anderson, W. F., *Science*, 256: 808-813, 1993;

Friedmann, T., *Science*, 244: 1275-1281, 1989). Preferred vectors include neurotropic vectors such as herpes simplex viral vectors (U.S. Patent No. 5,673,344 to Kelly et al.) and adenoviral vectors (Barkats et al., *Prog Neurobiol*, 55: 333-341, 1998).

In a further embodiment, present invention provides antibodies against PKMζ for detection, treatment and/or prevention of neurological disorders, e.g. AD.

The present invention further provides antibodies against PKMζ for detection, treatment and/or prevention of neurological disorders, e.g. AD. Those skilled in the art can use any of the well-known techniques and commercially available resources to generate such monoclonal or polyclonal antibodies (Harlow et al., Using Antibodies: A Laboratory Manual, 1999). Once an antibody is obtained, such antibody can be tested in assays to determine whether such antibody exhibits specific activity.

A still further embodiment of the present invention provides a method for constructing transgenic animals, such as a knock-out mouse lacking aPKC or a mouse that overexpresses the wild type or a mutant aPKC, that are useful as animal models of neurological dysfunction. The method includes:

a. constructing a transgenic animal having an altered aPKC amount, localization or activity, e.g., by deleting an aPKC gene in the animal using knock-out techniques known to one skilled in the art,
b. treating the transgenic animal from Step a with a candidate treatment process,
c. treating the transgenic animal from Step a with a control treatment process,
d. assaying the transgenic animals from Steps b and c for a biochemical or behavioral change, and
e. comparing the results of the assay of the transgenic animals from Steps b and Step c, wherein a difference is indicative the efficacy of the candidate treatment process in alleviating the neurological dysfunction.

A variation of the method involves over-expression of either wild-type or mutated aPKC in a transgenic animal, alone or in combination with one or more other transgenes expressing mutations or polymorphisms in genes associated with neurodegeneration, e.g. superoxide dismutase, tau, β-amyloid, α-synuclein, and apolipoprotein E.

A yet further embodiment of the present invention is directed to the genetic screening of DNA for mutations or polymorphisms in the DNA sequence of the aPKC genes, e.g. PKCζI/II and PKCι/λ, and regions regulating the expression of these genes including promoter regions, enhancer regions and negative regulatory regions. A method for such screening includes:

a. isolating DNA from a sample of a subject,
b. sequencing an aPKC gene or its regulating region of the DNA from Step a and,
c. comparing the DNA sequence from Step b to a reference DNA sequence, wherein the reference DNA is a known normal DNA sequence of the same region as sequenced DNA in Step b but without any mutation, whereby a difference between the DNA sequence from Step b and the reference sequence is indicative of genetic susceptibility to a neurological or psychiatric disorder.

A particular embodiment of the present invention is directed to the use of a known aPKC interacting protein for all of the above methods.

The present invention is also directed toward the diagnosis and treatment of other abnormal protein aggregates including α-synuclein seen in PD, multisystem atrophy (MSA) and dementia with Lewy bodies (DLB) and β-amyloid in AD and normal aging.

According to the present invention, abnormal aggregation of aPKC protein, such as PKCι/λ (one of the two members of aPKC) protein, can cause neurodegenerative disorders. It is a discovery of the present invention that PKCι/λ protein distribute in a variety of tauopathies and α-synucleinopathies. Specifically, using immunocytochemistry, the present invention demonstrates that an anti-PKCι/λ antibody can label tau-positive structures in Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and

Pick's disease (PiD); α-synuclein-positive Lewy bodies in idiopathic Parkinson's disease and dementia with Lewy bodies, and glial inclusions in multi-system atrophy; αB-crystalline-containing ballooned neurons in CBD and PiD; and actin-rich Hirano bodies in AD, PiD and elderly individuals. See Example 12.

Still another emobidment of the present invention provides methods for diagnosing various forms of cancer, such as neuroblastoma, oligodendroglioma, meningioma, lymphoma (myeloma), leukemia (including acute myelocytic leukemia (AML)), melanoma, squamous cell carcinoma, hepatocellular carcinoma, parathyroid tumors, pheochromocytoma, paraganglioma, intravascular lymphomatosis, breast cancer, liver cancer, lung cancer, prostate cancer, bladder cancer, ovarian cancer, endometrial cancer, head and neck cancer and gastrointestinal cancers including colorectal cancer and pancreatic cancer. According to the present invention, a method for diagnosing and/or staging a tumor is provided. The diagnostic method of the invention includes the steps of:

a. contacting a tumor sample, e. g. a human biopsy sample, from a subject, with an aPKC specific probe, preferably a PKCζ specific probe,
b. detecting the binding of the probe to the tumor sample to quantify the level, amount or activity of said aPKC in said tumor sample,
c. contacting a control sample with the aPKC specific probe,
d. detecting the binding of the probe to the control sample to quantify the level, amount or activity of the aPKC in the control sample, and
e. comparing the level, amount or activity of the aPKC in Step b with the level, amount or activity of the aPKC in Step d, wherein the difference of the level, amount or activity of the aPKC is indicative of the type and the staging of the tumor.

Another embodiment of the invention provides methods for drug screening for the purpose of treating and/or preventing cancer, e. g. neuroblastoma. According to the present invention, a method for screening molecular compounds, e. g. peptides and small molecules, is provided.

Still another embodiment of the invention provides methods for treating cancer with gene therapy.

According to the present invention, the therapeutic compositions provided herein, such as expression vectors or antibodies, can be administered to the subject being treated by standard routes, including, but not limited to, the oral, ophthalmic, nasal, topical, transdermal, parenteral (e.g., intravenous, intraperitoneal, intradermal, subcutaneous or intramuscular), intracranial, intracerebral, intraspinal, intravaginal, intrauterine, or rectal route. Depending on the condition being treated, one route may be preferred over others, which can be determined by those skilled in the art. For example, the topical or dermal route can be chosen when the target area includes tissues or organs readily accessible by topical application, such as neurological conditions of the eye or the facial tissue. For certain conditions, direct injection or surgical implantation in the proximity of the damaged tissues or cells is preferred in order to avoid the problems presented by the blood-brain barrier. Successful delivery to the central nervous system (CNS) by direct injection or implantation has been documented (Otto et al., J Neurosci Res, 22: 83-91, 1989; Goodman et al., Goodman & Gilman's the Pharmacological Basis of Therapeutics, 2001; Williams et al., Proc Natl Acad Sci USA, 83: 9231-35, 1986).

According to the present invention, the therapeutic ingredients are preferably administered to the subject in need thereof as early as possible, e.g., after the neuronal injury or neuronal death caused by NFT, or any other nervous system disorder or cancer occurs, in order to achieve the best therapeutic efficacy. The amount administered varies among patients and by route administered but should be sufficient enough to achieve a concentration to treat or prevent the disease or disorder or is sufficient to maintain said aPKC activity at a normal level. By "normal level" is meant that the level a biomolecule or compound functions or occurrs in a natural way; lacks observable or scientifically detectable abnormalities or deficiencies. For example, normal level aPKC in a human body or an organ or tissue thereof is from about 0.005% to about 0.05% of total protein in the human body or an organ or tissue thereof, preferably, the organ is a human brain. In a particular embodiment of the present invention, ZIP or chelerythrine can be administered between about 100 nM and about 10 μM of in a desired tissue.

The present invention is further illustrated by the following non-limiting example. This example is included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the

techniques disclosed in the example represents techniques discovered by the inventors to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention, therefore all matter set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense. All references cited herein are hereby expressly incorporated-herein by reference.

Example 1

Production of PKC isozyme antisera

Peptides used as immunogens were synthesized by Quality Controlled Biochemicals (Hopkinton, MA) and corresponded to the amino-terminal (ζ-N1, TDPKMDRSGGRVRLKC, SEQ ID NO: 1), catalytic-domain (ζ-C2, TLPPFQPQITDDYGLC, SEQ ID NO: 2) or carboxyl-terminal (ζ-C1, EYINPLLLSAEESV, SEQ ID NO: 3) of PKCζ. In addition, a terminal cysteine residue was added to each of the sequences for coupling purposes. The peptides were coupled to maleimide-activated bovine serum albumin (BSA, Pierce, Rockford, IL), according to the manufacturer's instructions. Peptide conjugates were mixed with Titermax (CytRx Corp., Norcross, GA) and injected intramuscularly into female New Zealand rabbits, one to three weeks of age. After three boosts at four-week intervals, the antisera were affinity-purified on Sulpholink gel columns (Pierce, Rockford, IL), to which the immunizing peptide had been conjugated according to the manufacturer's instructions. The antiserum to ι/λ is a mouse monoclonal antibody prepared against the ι/λ catalytic domain (Transduction Laboratories, Lexington, KY).

Example 2

Western blot of AD brain

Fresh frozen tissue from autopsy brain tissue derived from 4 neuropathologically confirmed cases of AD and 3 controls from individuals without neurological disease were homogenized in buffer containing protease inhibitors (50 mM HEPES, pH 7.5, 5 mM EDTA, 5 mM EGTA, 5 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, aprotinin (17 kallikrein units/ml), 5 mM benzamidine, 0.1 mM leupeptin) and phosphatase inhibitors (50 mM NaF, 40 mM β-glycerol phosphate, 10 mM pyrophosphate). Protein concentration was measured by Pierce assay. Sample buffer was added and samples boiled for 10 min. 15 μg of total protein was subjected to SDS-PAGE, transferred to nitrocellulose, and probed with specific anti-PKCζ and anti-PKCι/λ antibodies, and visualized by enhanced chemiluminescence (Amersham Biosciences, Freiburg, Germany).

Example 3

Pathological stains

Eight micron sections were cut from paraffin embedded tissue. All tissue was deparaffinized and rehydrated prior to use. For hematoxylin and eosin staining, sections were incubated in Gills hematoxylin for 5 minutes, rinsed in water, two changes, then dipped in bluing solution ten times and rinsed again. Next, sections were incubated in eosin Y for 4 minutes, dehydrated and coversliped. For Sevier-Munger silver staining, slides were incubated in 60 °C silver nitrate solution (20%) for 15 min. Slides were rinsed and placed in a clean dry staining jar. Slides were then developed in ammoniacal silver solution for 5 to 30 minutes. Sections were then rinsed 3 changes of water and incubated in 5% sodium thiosulfate for 2 minutes. Last, slides were washed with water, dehydrated, and cover-slipped.

Example 4

Antigen retrieval

For antigen retrieval, deparaffinized and rehydrated slides were submerged in 10 mM citrate buffer (pH 6.0) and microwaved for 5 minutes. Slides were then transferred to a second container with heated distilled water and allowed to cool. Slides were then rinsed in PBS for 5 minutes. Some slides were treated with formic acid for 3 minutes prior to citrate treatment.

Example 5

Immunohistochemical staining

Slides were treated with 3% hydrogen peroxide for 10 minutes, rinsed in PBS for 5 minutes, and blocked in 4% normal horse serum for 20 minutes. Tissue was then incubated in primary antibody in a humidity chamber overnight on a rotator plate. The following day, slides were rinsed in two changes of PBS for 10 minutes each and incubated in biotinylated secondary (1:200) for 30 min. Slides were then rinsed again in two changes of PBS for 5 minutes each then incubated in R.T.U. ABC reagent (Vector Laboratories) for 30 minutes, and rinsed again in two changes of PBS for 5 minutes each. Tissue was then developed in DAB solution, rinsed in tap water and counterstained with hematoxylin, dehydrated and coverslipped.

Example 6

PKMζ protein is present in human brain

Western blot on homogenates of human brain samples were conducted to confirm antisera specificity and document PKMζ protein in humans (Fig. 3). Fresh frozen tissue was obtained from the Rush Alzheimer's Disease Center (Chicago, Illinois) (Table 1). The average PMI for the AD (4.75 h) and control (4.00 h) cases was the same. The average age for the AD (69.5 yr) cases was less than controls (86.7 yr). The diagnosis was confirmed pathologically for all patients.

Table 1: Summary of patient data (Western blot)

Case	PMI (hours)	Age	Sex	Reagan Index	Braak Stage
1	3	85	F	Low likelihood of AD	I/II
2	4	87	M	Low likelihood of AD	I/II
3	5	88	F	Low likelihood of AD	I/II
4	4.5	78	M	High likelihood of AD	V/VI
5	5.5	62	F	High likelihood of AD	V/VI
6	4.5	64	F	High likelihood of AD	V/VI
7	4.5	74	M	High likelihood of AD	V/VI

The PKCι antibody showed one band at ~79 kD in all regions tested. A faint, minor band at ~50 kD was seen suggesting the presence of a small amount of PKMι. The C-terminal antibody, ζ-C1, which detects both atypical forms, detected three bands at ~55, ~79 and ~160 kD. These bands represent aPKM, aPKC

and an unidentified band respectively. The aPKC band was strongest in the cerebellum and weak in all other regions. The ζ specific antibody, ζ-C2, which does not cross-react with ι but reacts with both PKCζ and PKMζ detected major bands at ~55 kD and ~79 kD, and a minor band at ~60 kD. The ~79 kD band is PKCζ and was only present in the cerebellum. The 55 kD and 60 kD bands were seen in all regions tested. The N-terminal antibody, ζ-N1, which reacts only with ζ forms with a regulatory domain showed one strong unidentified band at ~45 kD. There were no PKM or PKC forms seen with this antibody.

Example 7

PKCγ and PKCι/λ but not PKM ζ decrease in the superior temporal cortex in AD

To determine whether there are changes in the levels of aPKC, densitometric analysis of Western blots was done on total protein homogenates prepared from the superior temporal cortex derived from AD (n=4) and controls (n=3). PKCγ was included for comparison. PKCγ was significantly lower (p = 0.031) in the AD samples (0.30 ± 0.07) than controls (0.79 ± 0.34) representing a 63% decrease. There was a smaller, but statistically significant decrease in PKCι (p = 0.006) in AD (0.86 ± 0.08) versus controls (1.15 ± 0.08) representing a 25% decrease. In contrast, PKMζ was not significantly different in AD (0.69 ± 0.39) versus controls (0.63 ± 0.22). See, Figure 4.

Example 8

PKCι/λ but not PKMζ nor PKCγ increase in the caudate nucleus in AD

For comparison, Western blots were conducted on the caudate nucleus. PKCγ was not significantly changed in the AD samples (1.44 ± 0.15) as compared to controls (1.24 ± 0.10). There was a smaller, but statistically significant decrease in PKCι ($p = 0.006$) in AD (0.86 ± 0.08) versus controls (1.15 ± 0.08) representing a 25% decrease. In contrast, PKMζ was not significantly different in AD (0.69 ± 0.39) versus controls (0.63 ± 0.22). See Figure 5.

Example 9

Localization of aPKC in human brain

To localize aPKC in human brain, immunohistochemistry was done on paraffin-embedded sections from control cases (n=2).

Table 2: Summary of patient data (immunohistochemistry)

Case	PMI	Age	Sex	Dx
1	4.5	82	F	AD
2	6.5	86	F	AD
3	4.0	72	F	AD
4	Na	60	F	AD
5	Na	83	F	AD
6	Na	98	M	Braak Stage II
7	12	47	F	control

Staining with ζ-C1 was evident in the perikarya of pyramidal cells, extending into proximal dendrites (Fig. 6B). There was no neuronal nuclear staining.

Staining was also observed in glia. The cytoplasm and nuclei of astrocytes in hippocampal area CA-4 stained strongly with ζ-C1 indicating the presence of aPKC (Fig. 7B). PKCι/λ immunoreactivity in astrocytes was also observed in cortex (Fig. 7A). ζ-C2 did not stain astrocytes.

Ependymal cells also stained with ζ-C1 (Fig. 8). Similar results were seen with ζ-C2 and ι/λ, indicating the presence of both ζ and ι/λ forms in this cell type.

Example 10

PKM ζ colocalizes with neurofibrillary tangles and Hirano bodies

To examine changes in the distribution of aPKC in AD, sections of hippocampus from patients (n=4) with neuropathologically confirmed AD were stained with aPKC antibodies. Despite high staining in the neuropil, there was minimal ι/λ immunoreactivity in NFTs (Fig. 9, top right). In contrast, both ζ-C1 and ζ-C2 strongly reacted with NFTs (Fig. 9, middle right and left). Silver stain confirms the presence of SPs and NFTs in this region. Neuropil threads, dystrophic neurites and SPs failed to label. Since neuropil threads and dystrophic neurites both contain PHFs, sections were pre-treated with citrate and formic acid to unmask the epitopes. Immunoreactivity in distrophic neurites and neuropil threads was seen after unmasking when stained with ζ-C1 (Fig. 10). Silver stain from a non-adjacent section in the same region confirmed the presence of SPs.

Strong staining was seen in HB with ζ-C1, ζ-C2 and ι/λ. On H&E, the presence of HB was confirmed. See Figure 11.

Example 11

The distribution of PKMζ correlates with that of a unique brain RNA encoding an independent ζ catalytic domain

One explanation for the abundance of PKMζ in forebrain despite very low levels of PKCζ is that PKMζ is not a proteolytic product of PKCζ. The ζ gene produces two sets of RNAs: full-length PKCζ mRNA and an RNA referred to as ζ' (Figure 12A). The 3' end of ζ' RNA consists of sequence for a partial ζ regulatory domain and its complete hinge and catalytic domain, which are identical to that in PKCζ mRNA (Figure 12A). The 5' end of ζ' RNA, however, is unique sequence that lacks an AUG to initiate translation of the ζ regulatory domain. The first AUG for an open reading frame (ORF) of the ζ sequence begins in its hinge (Figure 12A). Therefore, the ζ' RNA can express PKMζ. To determine whether the distributions of the ζ RNAs correlate with the different ζ proteins, the expression of PKCζ mRNA and ζ' was analyzed by RT-PCR, RNase protection, and Northern blot analysis (Figures 12A-12C).

For reverse-transcription polymerase chain reaction (RT-PCR), total RNA isolated from rat tissue was used to synthesize cDNA with the SuperScript Preamplification System for First Strand cDNA Synthesis Kit (Gibco Invitrogen, Grand Island, NY). 200 ng of cDNA was used in 100 µl final volume PCR reactions. Amplified was for 34 cycles with 94 °C for 30 sec, 60 °C for 1 minutes, and 72 °C for 1 minutes as cycle parameters, with a final step of 72 °C for 10 minutes. For amplification of PKCζ and PKMζ cDNAs, specific forward primers were F 5'-CCATGCCCAGCAGGACCACC-3' (SEQ ID NO: 12) and F 5'-CCTTCTATTAGATGCCTGCTCTCC-3' (SEQ ID NO: 13), respectively, and R 5'-TGAAGGAAGGTCTACACCATCGTTC-3' (SEQ ID NO: 14), was the reverse primer for both. As a control we used GAPDH primers, F 5'-ACATGGTCTACATGTTCC-3' (SEQ ID NO: 15) and R 5'-CAGATCCACAACGGAATAC-3' (SEQ ID NO: 16).

Using specific forward primers that distinguish between the two ζ RNAs, RT-PCR analysis showed abundant expression of ζ' in brain, but not in non-neural tissues (Figure 12A), in accordance with the distribution of PKMζ. Only with a higher number of PCR cycles could a small amount of ζ' RNA be detected in kidney (data not shown). In contrast, PKCζ mRNA was expressed in kidney, lung, testis, and

cerebellum, but not in neocortex or hippocampus (Figure 12A). This distribution correlates with the expression of PKCζ.

These findings by RNase protection were quantified using an antisense probe that protects a 345 nucleotide fragment of PKCζ mRNA and a 202 nucleotide fragment of ζ' RNA (Figure 12B). Confirming the RT-PCR, the RNase protection product of ζ' RNA was found only in brain, whereas the protection product of the PKCζ mRNA was observed in kidney, lung, testis, and cerebellum, but not in neocortex or hippocampus (Figure 12B). The relative levels of PKCζ mRNA and ζ' RNA were examined by comparing them to mRNA for the housekeeping rat acidic ribosomal protein (RARP, Figure 12B, center and bottom). The expression of ζ' RNA in brain was higher than that of PKCζ mRNA in any of the tissues examined.

For Northern blot, total RNA from rat tissue (30 ug) was electrophoresed and transferred to nitrocellulose, rinsed, and UV cross-linked. Digestion of rat cDNAs with EcoRI-SphI and KpnI-EcoRI gave a 457 bp and 227 bp specific fragment for PKCζ and PKMζrespectively. The fragments were radiolabeled with ^{32}P using a Stratagene random octamer protocol (Stratagene Cloning Systems, La Jolla, CA). Hybridization conditions were performed according to instructions for Stratagene QuickHyb Hybridization. Blots were developed overnight by film exposure at –70 °C or by PhosphorImager (Molecular Dynamics Storm 860 gel and blot imaging system, Amersham Pharmacia Biotech, Piscataway, NJ). The sizes of the ζ' RNA and PKCζ mRNAs in different brain regions and kidney were then determined by Northern blot, using probes specific to their unique 5' ends (Figure 12C). The ζ' RNA was expressed as a 2.3 kb and 4.7 kb species in brain, but not kidney (Figure 12C). PKCζ mRNA was expressed as a 2.5 kb and minor 4.8 kb species in kidney and cerebellum, but not in neocortex or hippocampus (Figure 12C). These sizes were similar to those previously reported.

EXAMPLE 12

In all brains, whether those of neurologically normal individuals or those of patients with neurodegenerative diseases, the PKCι/λ antibody showed widespread weak, fine granular labeling of neuronal cell bodies and neuropil in cortex, subcortical gray matter, brainstem and cerebellum (Fig. 14a). The antibody consistently labeled ependymal cells and choroid plexus epithelial cells, but glial cells were immunonegative (data not shown).

In AD, the PKCι/λ antibody strongly labeled a subset of NFTs in hippocampal pyramidal cells and neocortical neurons (Fig. 14b) as well as most Hirano bodies in CA1 of the hippocampus (Fig. 14c). PKCι/λ-positive NFTs generally exhibited classic flame-shaped configurations. Interestingly, while 'early' perinuclear tangles were also labeled, the more advanced 'ghost' or 'extracellular' tangles failed to label. Neuropil threads, amyloid plaques and granulovacuolar bodies in hippocampal neurons were generally immunonegative.

The PKCι/λ antibody uniformly labeled Pick bodies and occasional Pick cells in neurons of hippocampal dentate gyrus and neocortex of PiD (Fig. 14d). In cases of PSP, the antibody labeled globose tangles and tufted astrocytes in the subthalamic, mesencephalic, inferior olivary and cerebellar dentate nuclei (Figs. 14e-14f). In CBD, ballooned neurons, tau-immunoreactive astrocytic inclusions in cerebral cortex, as well as neuronal and glial inclusions in the basal ganglia were immunopositive (Figs. 14g-14h).

In the α-synucleinopathies, the PKCι/λ antibody strongly labeled all classic Lewy bodies found in the substantia nigra as well as the majority of Lewy bodies in cerebral cortex and amygdala in PD and DLB (Figs. 14i-14j). Lewy neurites in amygdala, hippocampal CA2/3 or midbrain were labeled only rarely. The PKCι/λ antibody also labeled some glial α-synuclein-positive inclusions in MSA (Fig. 14k). Omission of the primary antibody resulted in no immunolabeling of any of these structures (Fig. 14l).

EXAMPLE 13

Single nucleotide polymorphisms were identified in the PKCζ and PKCι/λ genes (SEQ ID NOs: 6 and 7 respectively). The sequences obtained using the genome browser (UCSC, CA) and examined for SNPs from clone overlaps and SNPs from random reads. SNPs identified in the PKCζ and PKCι/λ genes are indicated in the sequence in bold letters. For reference, exons are indicated in capital letters.

Sequence Listing

SEQ ID NO: 1

TDPKMDRSGGRVRLKC

SEQ ID NO: 2

TLPPFQPQITDDYGLC

SEQ ID NO: 3

EYINPLLLSAEESV

SEQ ID NO: 4 Homo sapiens, protein kinase C, zeta I, clone MGC:10512 mRNA, complete cds

GGCACGAGGGCCCCGCGCGCCGCCGGAGTTCCGCGGAGTTGACCGGGTC
GGCGCCGTCGGTCCTGAGCGCTGCCTTCCGCGTTCCGCCGCGGCCCCACC
TGGAGCCCCCGCCCCGCGCCATGGCCGGAGCTCCCGGGGCGCAGCGCTG
ACGGCGGCGGGGGGAGCGCGCCATGCCCAGCAGGACCGGCCCCAAGAT
GGAAGGGAGCGGCGGCCGCGTCCGCCTCAAGGCGCATTACGGGGGGGA
CATCTTCATCACCAGCGTGGACGCCGCCACGACCTTCGAGGAGCTCTGTG
AGGAAGTGAGAGACATGTGTCGTCTGCACCAGCAGCACCCGCTCACCCT
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GAGCTGGAAGAGGCTTTCCGCCTGGCCCGTCAGTGCAGGGATGAAGGCC
TCATCATTCATGTTTTCCCGAGCACCCCTGAGCAGCCTGGCCTGCCATGT
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TTCCGAGGAGACAGATGGAATTGCTTACATTTCCTCATCCCGGAAGCATG
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TGCCATAAAGAGGATCGACCAGTCAGAGTTCGAAGGCTTTGAGTATATC
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CTGTCGTGGACACGCGTGATTGACCCTTTAACTGTATCCTTAACCACCGC
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GCTTGCGCCGAGACCGCAGAGGGAAGCGTCAGCGGGCGCTGCTGGGAG
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GGAACTTGCTGCTGTTCCTGCGTCGCGGCGGATCCGCGGGGACCCTGCC
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TGAGGAATAAAATGTTCCGATGAAAAAAAAAAAAAAAAAAAAAA

SEQ ID NO: 5 mRNA sequences: PKCzeta II (PKMzeta) mRNA and protein

Sequence Range: 1 to 2252

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CGAUGUCGCA UUUUCAAGGU CCGCUGAGUC CGAGCCCUGC CUGGGUCUGG

        60         70         80         90        100
CUGCUGCCCG CCCGCUCUCU GGACUGUGCU GAUGCAGAGA UGCUUGUUUU

       110        120        130        140        150
CCUGUGACGU CAGCGUCAGC UCCUGCACAU CCAUGCCGUG UUUUAGUUUG

       160        170        180        190        200
UGCCUCAGCU GCUGGCUACA GCUUCCCGGG GGAGCCGGGU ACCACCCGGG
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        210         220         230         240         250
CCUGGAGACA UGAGGAGGCA GGGAUGUGAG GGGCGGGGGA CAGGACAGCC

        260         270         280         290         300
GGCCUUCCGU UAAAUAUCUG CUCCUCGCGC UCGAGCCUCC CUGCCUAUUG

        310         320         330         340         350
UCGGGGCCGG AGGCGAGCCG ACGCAGCAUC AGCUCGUCAA CGGGAAGGAA

        360         370         380         390         400
GAUGCCUCCC UGCACGCCCG CCGCGCACAG AGCAUAAAGA AUCUGCGCUG

        410         420         430         440         450
AGGAGGCAGG AGAAGAAAGC CGAAUCUAUC UACCGCCGGG GAGCCAGAAG

        460         470         480         490         500
AUCGAGGAAG CUGUACCGUG CCAACGGCCA CCUCUUCCAA GCCAAGCGCU

        510         520         530         540         550
UUAACAGGAG AGCGUACUGC GGUCAGUGCA GCGAGAGGAU AUGGGGCCUC

        560         570         580         590         600
GCGAGGCAAG GCUACAGGUG CAUCAACUGC AAACUGCUGG UCCAUAAGCG

        610         620         630         640         650
CUGCCACGGC CUCGUCCCGC UGACCUGCAG GAAGCAUAUG GAUUCUGUCA
                                              M   D   S   V>
                                            ___a___________>

        660         670         680         690         700
UGCCUUCCCA AGAGCCUCCA GUAGACGACA AGAACGAGGA CGCCGACCUU
M   P   S   Q     E   P   P     V   D   D     K   N   E   D     A   D   L>
___________a_TRANSLATION OF HSPKMZMRNA [A]__a___________>

        710         720         730         740         750
CCUUCCGAGG AGACAGAUGG AAUUGCUUAC AUUUCCUCAU CCCGGAAGCA
  P   S   E     E   T   D   G     I   A   Y     I   S   S     S   R   K   H>
___________a_TRANSLATION OF HSPKMZMRNA [A]__a___________>

        760         770         780         790         800
UGACAGCAUU AAAGACGACU CGGAGGACCU UAAGCCAGUU AUCGAUGGGA
  D   S   I     K   D   D     S   E   D   L     K   P   V     I   D   G>
___________a_TRANSLATION OF HSPKMZMRNA [A]__a___________>

        810         820         830         840         850
UGGAUGGAAU CAAAAUCUCU CAGGGGCUUG GGCUGCAGGA CUUUGACCUA
M   D   G   I     K   I   S     Q   G   L     G   L   Q   D     F   D   L>
___________a_TRANSLATION OF HSPKMZMRNA [A]__a___________>

        860         870         880         890         900
AUCAGAGUCA UCGGGCGCGG GAGCUACGCC AAGGUUCUCC UGGUGCGGUU
  I   R   V     I   G   R   G     S   Y   A     K   V   L     L   V   R   L>
___________a_TRANSLATION OF HSPKMZMRNA [A]__a___________>

        910         920         930         940         950
GAAGAAGAAU GACCAAAUUU ACGCCAUGAA AGUGGUGAAG AAAGAGCUGG
  K   K   N     D   Q   I     Y   A   M   K     V   V   K     K   E   L>
___________a_TRANSLATION OF HSPKMZMRNA [A]__a___________>
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960 970 980 990 1000
UGCAUGAUGA CGAGGAUAUU GACUGGGUAC AGACAGAGAA GCACGUGUUU
V H D D E D I D W V Q T E K H V F>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1010 1020 1030 1040 1050
GAGCAGGCAU CCAGCAACCC CUUCCUGGUC GGAUUACACU CCUGCUUCCA
E Q A S S N P F L V G L H S C F Q>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1060 1070 1080 1090 1100
GACGACAAGU CGGUUGUUCC UGGUCAUUGA GUACGUCAAC GGCGGGGACC
T T S R L F L V I E Y V N G G D>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1110 1120 1130 1140 1150
UGAUGUUCCA CAUGCAGAGG CAGAGGAAGC UCCCUGAGGA GCACGCCAGG
L M F H M Q R Q R K L P E E H A R>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1160 1170 1180 1190 1200
UUCUACGCGG CCGAGAUCUG CAUCGCCCUC AACUUCCUGC ACGAGAGGGG
F Y A A E I C I A L N F L H E R G>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1210 1220 1230 1240 1250
GAUCAUCUAC AGGGACCUGA AGCUGGACAA CGUCCUCCUG GAUGCGGACG
I I Y R D L K L D N V L L D A D>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1260 1270 1280 1290 1300
GGCACAUCAA GCUCACAGAC UACGGCAUGU GCAAGGAAGG CCUGGGCCCU
G H I K L T D Y G M C K E G L G P>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1310 1320 1330 1340 1350
GGUGACACAA CGAGCACUUU CUGCGGAACC CCGAAUUACA UCGCCCCCGA
G D T T S T F C G T P N Y I A P E>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1360 1370 1380 1390 1400
AAUCCUGCGG GGAGAGGAGU ACGGGUUCAG CGUGGACUGG UGGGCGCUGG
I L R G E E Y G F S V D W W A L>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1410 1420 1430 1440 1450
GAGUCCUCAU GUUUGAGAUG AUGGCCGGGC GCUCCCCGUU CGACAUCAUC
G V L M F E M M A G R S P F D I I>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1460 1470 1480 1490 1500
ACCGACAACC CGGACAUGAA CACAGAGGAC UACCUUUUCC AAGUGAUCCU
T D N P D M N T E D Y L F Q V I L>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

1510 1520 1530 1540 1550
GGAGAAGCCC AUCCGGAUCC CCCGGUUCCU GUCCGUCAAA GCCUCCCAUG
E K P I R I P R F L S V K A S H>
_____a_TRANSLATION OF HSPKMZMRNA [A]__a_____>

```
      1560        1570        1580        1590        1600
UUUUAAAAGG AUUUUUAAAU AAGGACCCCA AAGAGAGGCU CGGCUGCCGG
V  L  K  G   F  L  N   K  D  P   K  E  R  L  G  C  R>
__________a_TRANSLATION OF HSPKMZMRNA [A]__a__________>

      1610        1620        1630        1640        1650
CCACAGACUG GAUUUUCUGA CAUCAAGUCC CACGCGUUCU UCCGCAGCAU
 P  Q  T   G  F  S  D   I  K  S   H  A  F   F  R  S  I>
__________a_TRANSLATION OF HSPKMZMRNA [A]__a__________>

      1660        1670        1680        1690        1700
AGACUGGGAC UUGCUGGAGA AGAAGCAGGC GCUCCCUCCA UUCCAGCCAC
  D  W  D   L  L  E   K  K  Q  A   L  P  P   F  Q  P>
__________a_TRANSLATION OF HSPKMZMRNA [A]__a__________>

      1710        1720        1730        1740        1750
AGAUCACAGA CGACUACGGU CUGGACAACU UUGACACACA GUUCACCAGC
Q  I  T  D   D  Y  G   L  D  N   F  D  T  Q   F  T  S>
__________a_TRANSLATION OF HSPKMZMRNA [A]__a__________>

      1760        1770        1780        1790        1800
GAGCCCGUGC AGCUGACCCC AGACGAUGAG GAUGCCAUAA AGAGGAUCGA
 E  P  V   Q  L  T  P   D  D  E   D  A  I   K  R  I  D>
__________a_TRANSLATION OF HSPKMZMRNA [A]__a__________>

      1810        1820        1830        1840        1850
CCAGUCAGAG UUCGAAGGCU UUGAGUAUAU CAACCCAUUA UUGCUGUCCA
  Q  S  E   F  E  G   F  E  Y  I   N  P  L   L  L  S>
__________a_TRANSLATION OF HSPKMZMRNA [A]__a__________>

      1860        1870        1880        1890        1900
CCGAGGAGUC GGUGUGAGGC CGCGUGCGUC UCUGUCGUGG ACACGCGUGA
T  E  E  S   V>
___TRANSLAT____>

      1910        1920        1930        1940        1950
UUGACCCUUU AACUGUAUCC UUAACCACCG CAUAUGCAUG CCAGGCUGGG

      1960        1970        1990        1980        2000
CACGGCUCCG AGGGCGGCCA GGGACAGACG CUUGCGCCGA GACCGCAGAG

      2010        2020        2030        2040        2040
GGAAGCGUCA GCGGGCGCUG CUGGGAGCAG AACAGUCCCU CACACCUGGC

      2060        2070        2080        2090        2100
CCGGCAGGCA GCUUCGUGCU GGAGGAACUU GCUGCUGUGC CUGCGUCGCG

      2110        2120        2130        2140        2150
GCGGAUCCGC GGGGACCCUG CCGAGGGGGC UGUCAUGCGG UUUCCAAGGU

      2160        2170        2180        2190        2200
GCACAUUUUC CACGGAAACA GAACUCGAUG CACUGACCUG CUCCGCCAGG

      2210        2220        2230        2240        2250
AAAGUGAGCG UGUAGCGUCC UGAGGAAUAA AAUGUUCCGA UGAAAAAAAA

AA
```

SEQ ID NO: 6 Genomic sequence for protein kinase C iota/lambda (human), (exons for PKC iota/lambda in capital letters and single nucleotide polymorphisms are indicated in bold)

>chr3:171261193-171342120
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SEQ ID NO: 7 Genomic sequence for PKC zeta I/PKC zeta II (protein kinase M zeta) gene from human. PKC zeta I and PKC zeta II are alternative transcripts from the same gene.
(exons for PKC zeta in capital letters and single nucleotide polymorphisms are indicated in bold)

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SEQ ID NO: 8 non-conventional ATP binding site motif, wherein G is amino acid glycine, X is any amino acid

GXGXXA

SEQ ID NO: 9 ATP binding site hallmark motif, wherein G is amino acid glycine, X

is any amino acid

GXGXXG

SEQ ID NO: 10 A zeta-inhibitory peptide (ZIP): myristoylated PKCζ pseudosubstrate peptide

SIYRRGARRWRKLY

SEQ ID NO: 11 Human PKC zeta I amino acid sequence

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SEQ ID NO: 16
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WHAT IS CLAIMED IS:

1. A method for diagnosing a nervous system disorder in a subject comprising:
    a. contacting a sample from said subject with an aPKC specific probe,
    b. detecting the binding of said probe to said sample to determine the activity of said aPKC in said sample,
    c. contacting a control sample with said aPKC specific probe,
    d. detecting the binding of said probe to said control sample to determine the activity of said aPKC in said control sample, and
    e. comparing the activity of said aPKC in Step b with the activity of said aPKC in Step d, wherein if the activity of said aPKC in Step b is different from the activity of said aPKC in Step d, a nervous system disorder in said subject is present.
2. The method of Claim 1, wherein Step d is performed by quantifying a label attached to said probe.
3. The method of Claim 1, wherein said disorder is a neurodegenerative, neurological or psychiatric disorder.
4. The method of Claim 1, wherein said probe is an antibody or a nucleic acid sequence.
5. The method of Clam 1, wherein said sample is a human tissue or tissue extract or body fluid.
6. The method of Clam 5, wherein said tissue is postmortem autopsy tissue or brain biopsy tissue.
7. The method of Claim 1, wherein said nervous system disorder is selected from the group consisting of Alzheimers disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Parkinson's disease (PD) and multisystem atrophy (MSA).
8. A method for identifying a compound useful for modulating the activity of an aPKC comprising:
    a. providing cells wherein said aPKC gene is expressed,
    b. incubating said cells with a candidate compound for a sufficient time to induce a change in the activity of aPKC in said cells,
    c. incubating said cells as in Step b, in the absence of said candidate compound or in the presence of a control compound,

d. contacting said cells from Step b and Step c with an equal amount of an aPKC-specific probe,

e. detecting the binding of said probe to said cells to determine the activity of said aPKC in said cell from Step b and Step c, and

f. comparing the activity of said aPKC in said cells, wherein if the activity of said aPKC in said cells from Step b is different from the activity of said aPKC in said cells from Step c, said candidate compound modulates aPKC.

9. The method of Claim 8, wherein said compound is a peptide, a small molecule or a macromolecule.

10. An *in vitro* method for identifying a compound useful for modulating the activity of an aPKC comprising:

a. providing cells wherein aPKC gene is expressed,

b. incubating an aPKC in a first container with adenosine triphosphate (ATP), a substrate of said aPKC and a candidate compound, wherein the phosphate of said ATP is radioactively labeled, for a sufficient time to induce a change in the activity of aPKC in said cells,

c. incubating said aPKC in a second container with ATP and said substrate of said aPKC, wherein the phosphate of said ATP is radioactively labeled, for a sufficient time to induce a change in the activity of aPKC in said cells

d. quantifying the amount of incorporation of the radioactively-labeled phosphate into said substrate in said first container and said second container,

e. comparing the amount of incorporation in said first container and said second container, whereby a difference in the amount of incorporation in said first container and said second container inindicates that said candidate compound is identified as a compound modulating aPKC activity.

11. The method of Claim 10, wherein said substrate is a protein tau or GSK3β.

12. A method for treating a nervous system disorder in a subject comprising:

a. inserting an aPKC sequence into an expression vector, and

b. administering said vector from Step a to said subject.

13. A method for preventing a nervous system disorder in a subject, comprising:
    a. inserting the aPKC sequence into an expression vector, and
    b. administering said vector from Step a to said subject.
14. The method of Claims 12-13, wherein said expression vector is administered via intraventricular, intravenous, inhalation, dermal or oral route.
15. The method of Claims 12-13, wherein the dosage to be administered is sufficient to maintain said aPKC activity at a normal level.
16. The method of Claim 15, wherein said normal level is an amount of said aPKC from about 0.005% to about 0.05% of total protein in a sample from said subject.
17. The method of Claim 13, wherein said method comprises gene therapy.
18. The method of any of the Claims 1, 13-14, wherein said disorder is characterized by abnormal aPKC activity.
19. The method of Claim 18, wherein said abnormal aPKC activity comprises phosphorylation of protein tau and co-localization with neurofibrillary tangles (NFT).
20. The method of any one of Claims 1, 13-14, wherein said disorder is selected from the group consisting of Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Parkinson's disease (PD) and multisystem atrophy (MSA).
21. A method of any one of Claims 1-19, wherein said aPKC is PKMζ.
22. An antibody which binds to an isoform of an aPKC molecule or a functional derivative thereof.
23. The antibody of Claim 22, wherein said aPKC is PKMζ
24. A method for treating a nervous system disorder of a subject comprising:
    a. generating an antibody against PKMζ, and
    b. administering said antibody or a functional fragment thereof to said subject.
25. A method for constructing an animal model of neurological dysfunction comprising:
    a. constructing a transgenic animal having an altered aPKC amount, localization or activity,
    b. treating said transgenic animal from Step a with a candidate

compound

c. treating said transgenic animal from Step a with a control compound,
d. assaying said transgenic animals from Steps b and c for a biochemical or behavioral changes, and
e. comparing the results from Step d, wherein differences are indicative the efficacy of said candidate compound in treatment of said neurological dysfunction.

26. The method of Claim 25, wherein said transgenic animal is a knock-out mouse lacking aPKC.
27. The method of Claim 25, wherein said transgenic animal is a mouse that overexpresses an aPKC.
28. The method of any of Claims 25-27, wherein said aPKC is a wild type or mutant aPKC
29. The method of Claim 25, wherein said biochemical change is tau phosphorylation
30. The method of Claim 25, wherein said behavioral change is a memory deficit.
31. A method for screening DNA for a mutation or polymorphisms in the DNA sequence of the aPKC genes and regions regulating the expression of these genes comprising:
    a. isolating DNA from a sample of a subject,
    b. sequencing said DNA, and
    c. comparing said sequence to a reference DNA sequence, whereby a difference between the DNA sequence derived from said subject and the reference sequence is indicative of genetic susceptibility to a neurological or psychiatric disorder.
32. The method of Claim 31, wherein Step c is performed by comparing single nucleotide polymorphisams (SNPs) on said sequence to SNPs on said reference DNA sequence.
33. The method of Claim 31, wherein said reference DNA sequence is a human genomic sequence of PKC iota/lambada set forth in SEQ ID NO: 6.
34. The method of Claim 31, wherein said reference DNA sequence is a human genomic sequence of PKC zeta set forth in SEQ ID NO: 7.

35. A method for diagnosing cancer comprising:
    a. contacting a tumor sample from a subject with an aPKC specific probe,
    b. detecting the binding of said probe to said tumor sample to quantify the activity of said aPKC in said tumor sample,
    c. contacting a control sample with said aPKC specific probe,
    d. detecting the binding of said probe to said control sample to quantify the activity of said aPKC in said control sample, and
    e. comparing said activity of said aPKC in Step b with said activity of said aPKC in Step d, wherein the difference of the activity of said aPKC is indicative of the type or the staging of said cancer.
36. The method of Claim 35, wherein said cancer is selected from the group consisting of neuroblastoma, oligodendroglioma, meningioma, lymphoma (myeloma), leukemia, melanoma, squamous cell carcinoma, hepatocellular carcinoma, parathyroid tumors, pheochromocytoma, paraganglioma, intravascular lymphomatosis, breast cancer, liver cancer, lung cancer, prostate cancer, bladder cancer, ovarian cancer, endometrial cancer, head and neck cancer, colorectal cancer and pancreatic cancer.
37. The method of Claims 35-36, wherein said method comprises gene therapy.

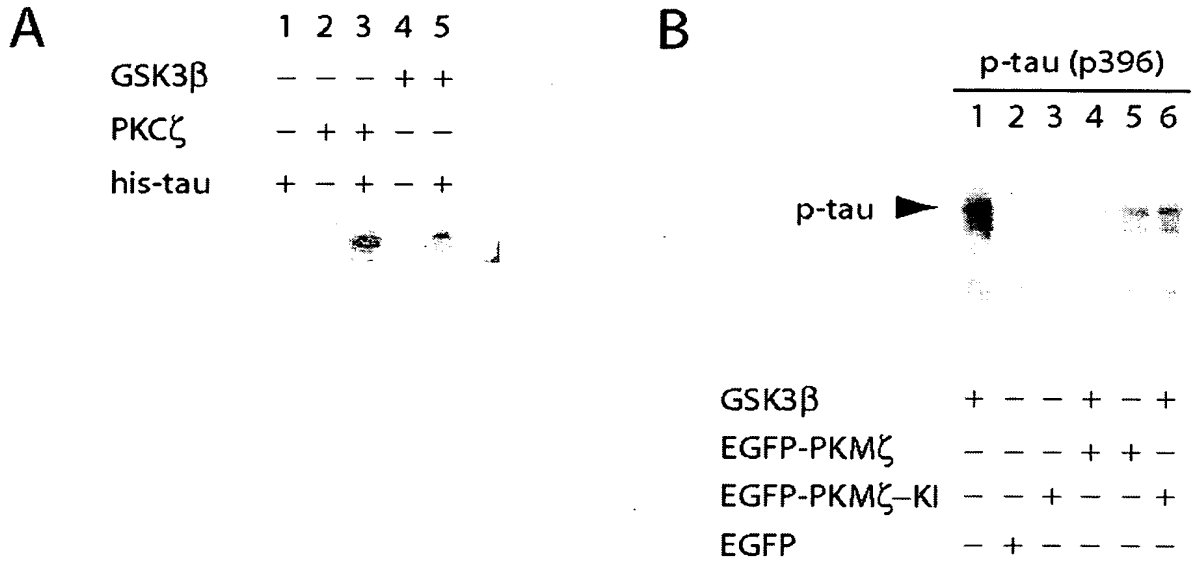
A
1 2 3 4 5
GSK3β – – – + +
PKCζ – + + – –
his-tau + – + – +
B
p-tau (p396)
1 2 3 4 5 6
p-tau
GSK3β + – – + – +
EGFP-PKMζ – – – + + –
EGFP-PKMζ–KI – – + – – +
EGFP – + – – – –

FIGURE 1

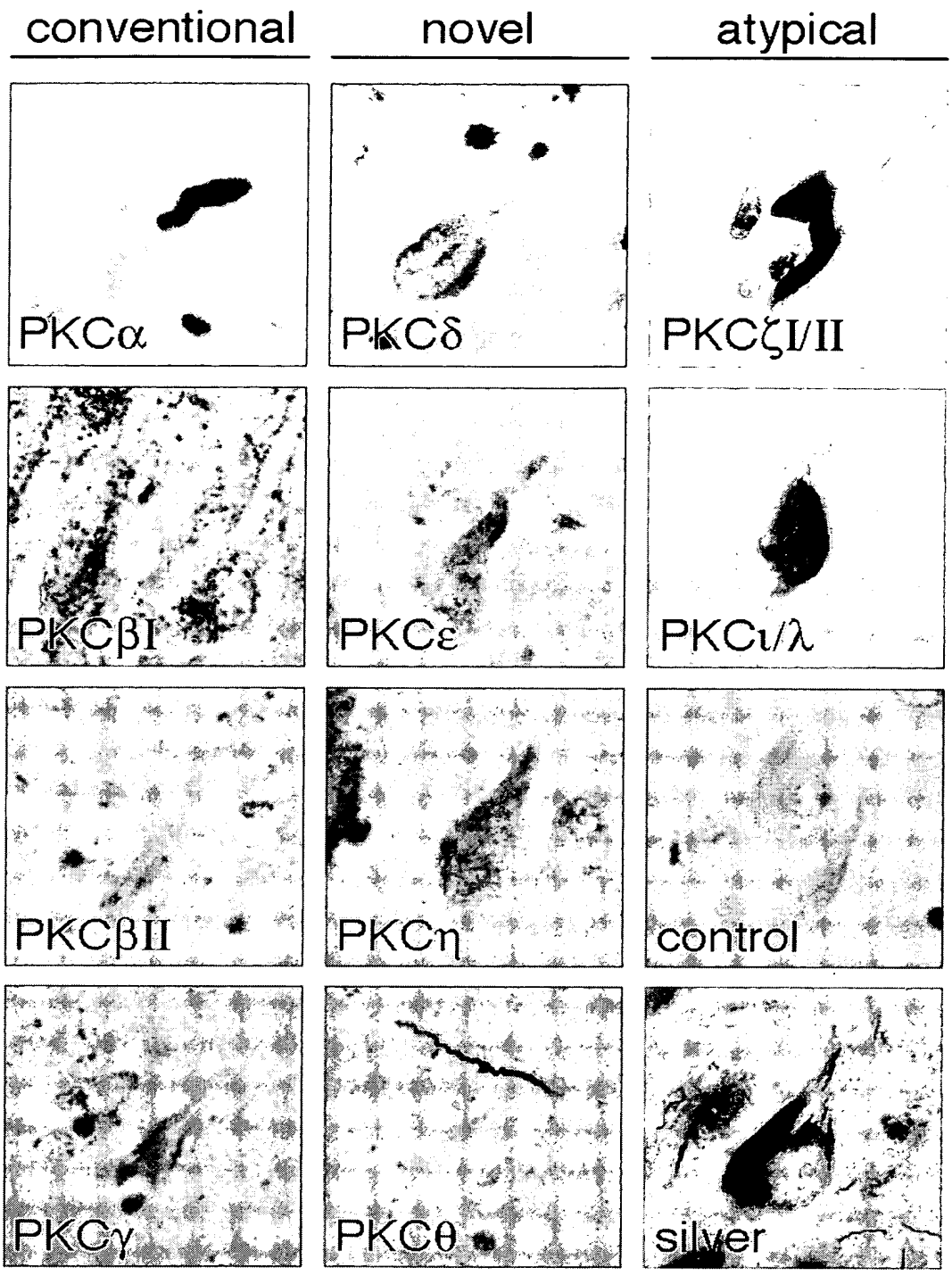

FIGURE 2

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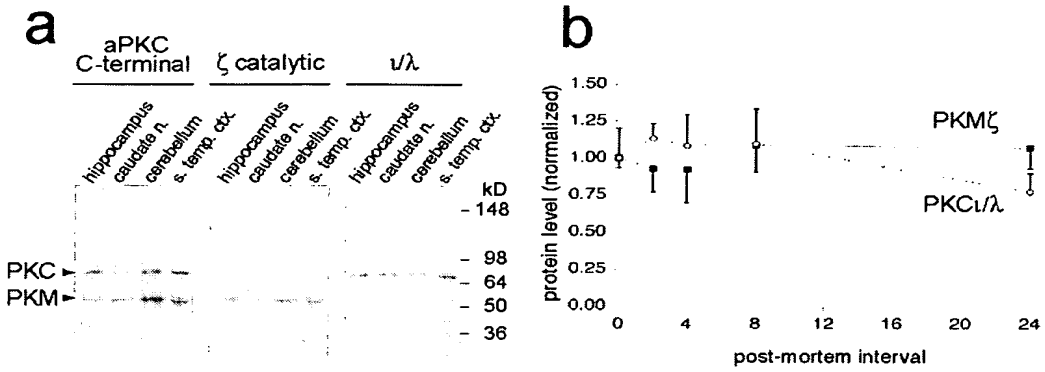
a
aPKC
C-terminal
ζ catalytic
ι/λ
hippocampus
caudate n.
cerebellum
s. temp. ctx.
kD
148
98
64
50
36
PKC
PKM
b
protein level (normalized)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
4
8
12
16
20
24
post-mortem interval
PKMζ
PKCι/λ

FIGURE 3

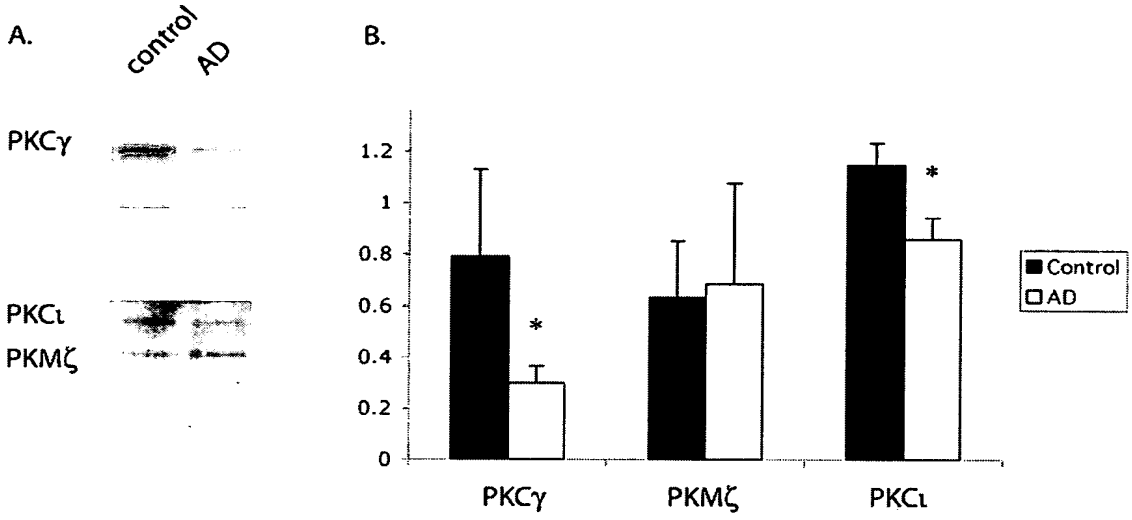

FIGURE 4

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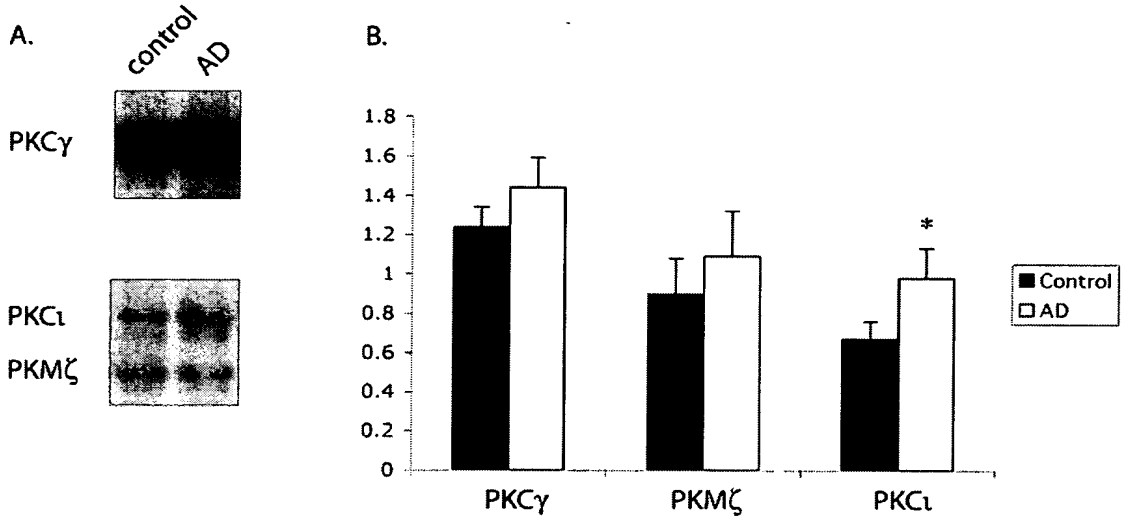
A.
control
AD
PKCγ
PKCι
PKMζ
B.
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Control
AD
PKCγ
PKMζ
PKCι

FIGURE 5

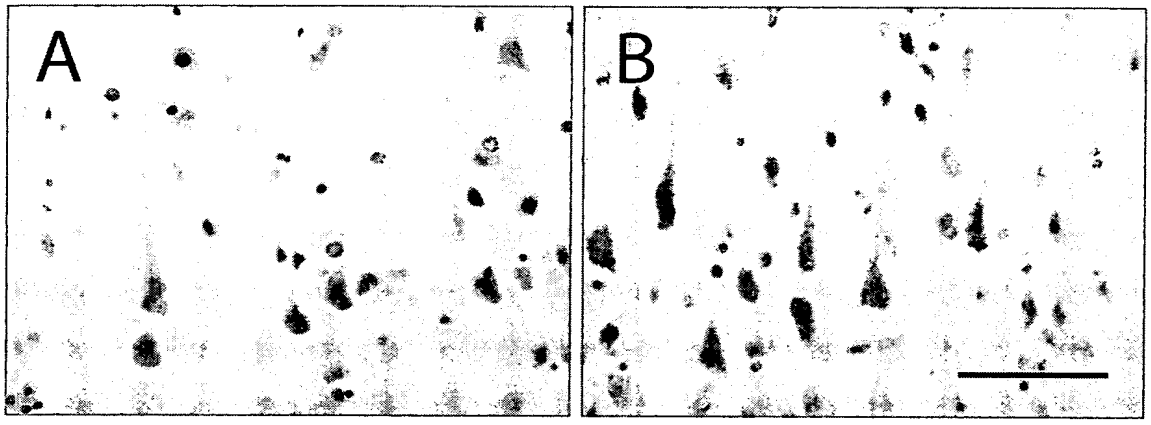

FIGURE 6

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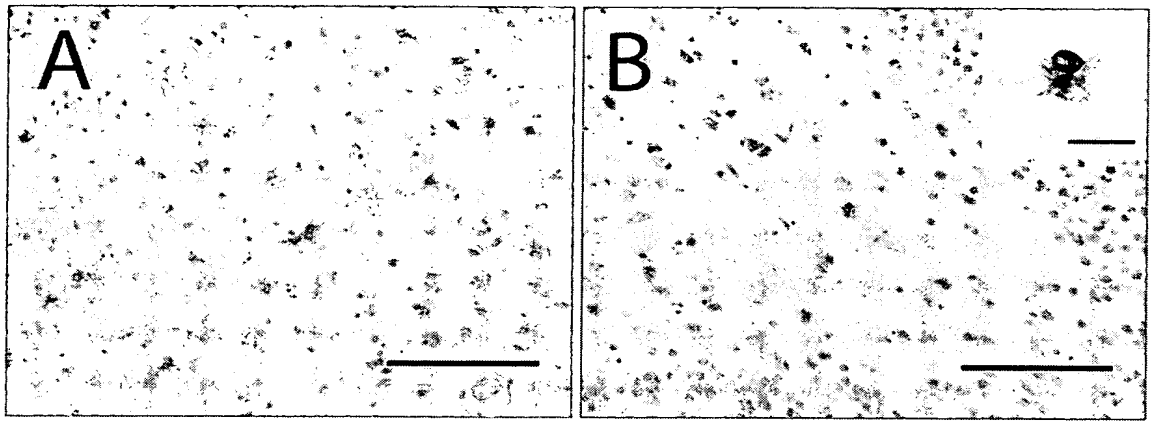

FIGURE 7

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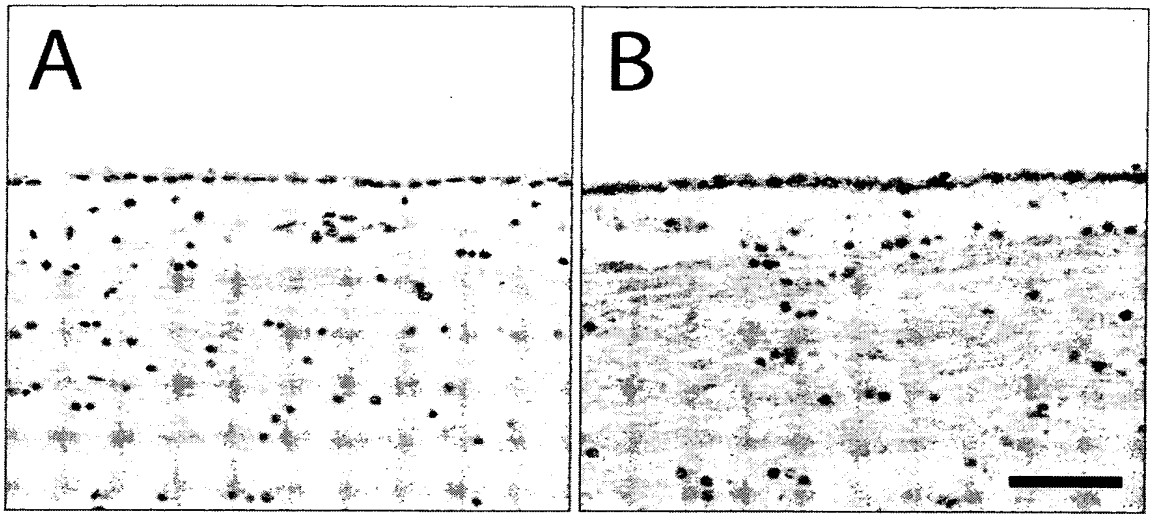

FIGURE 8

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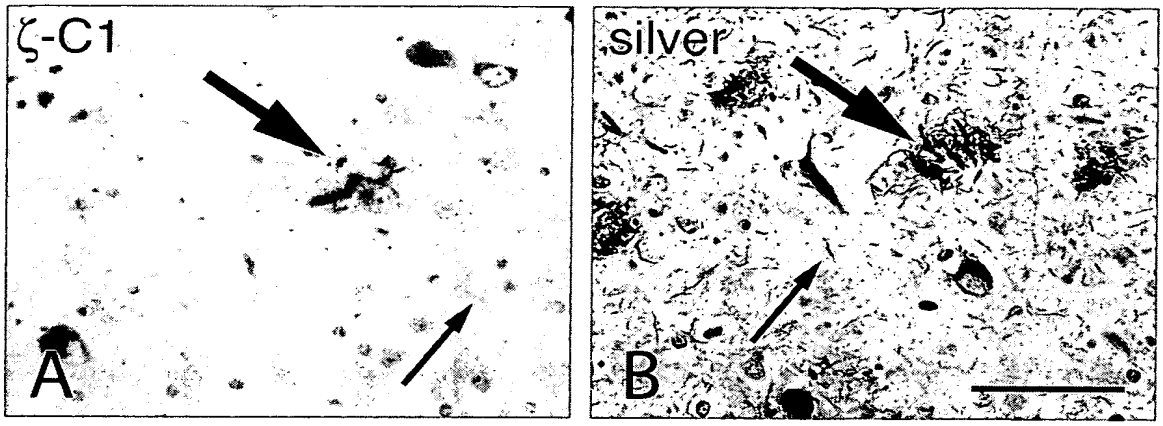

FIGURE 9

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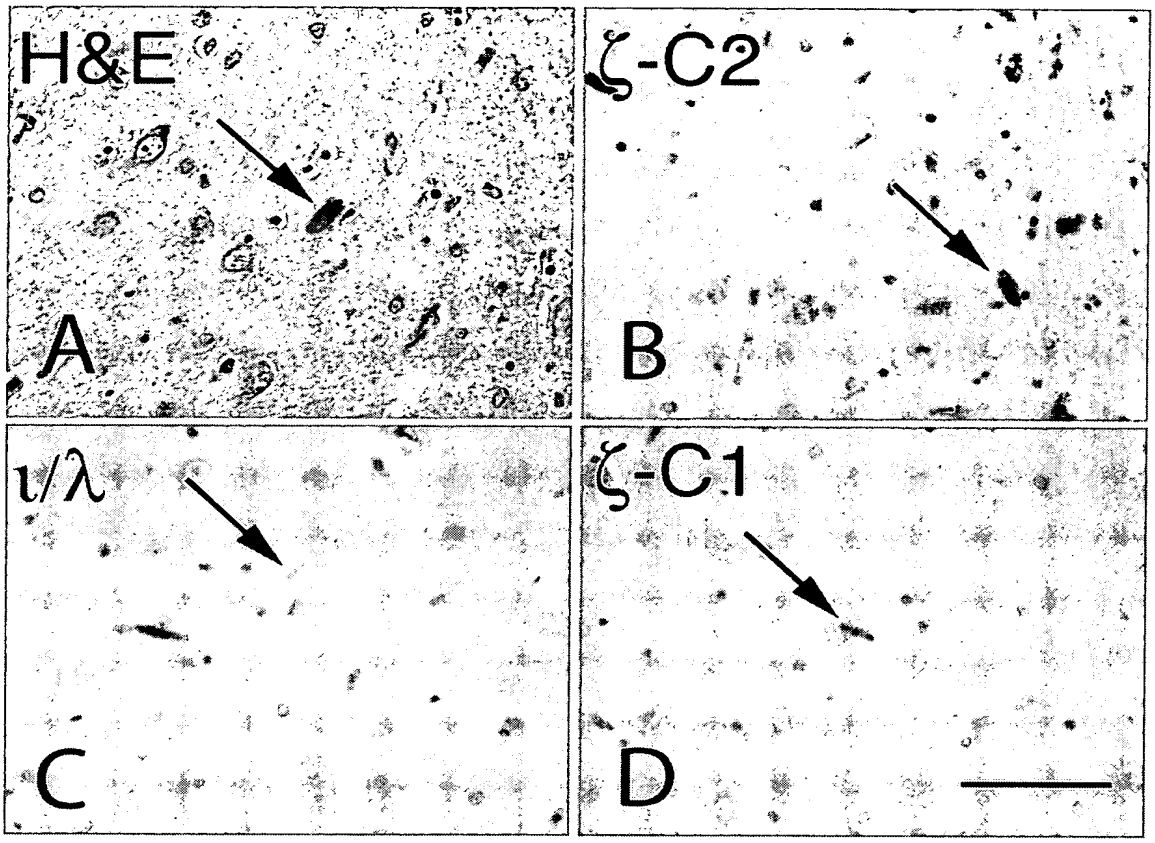

FIGURE 10

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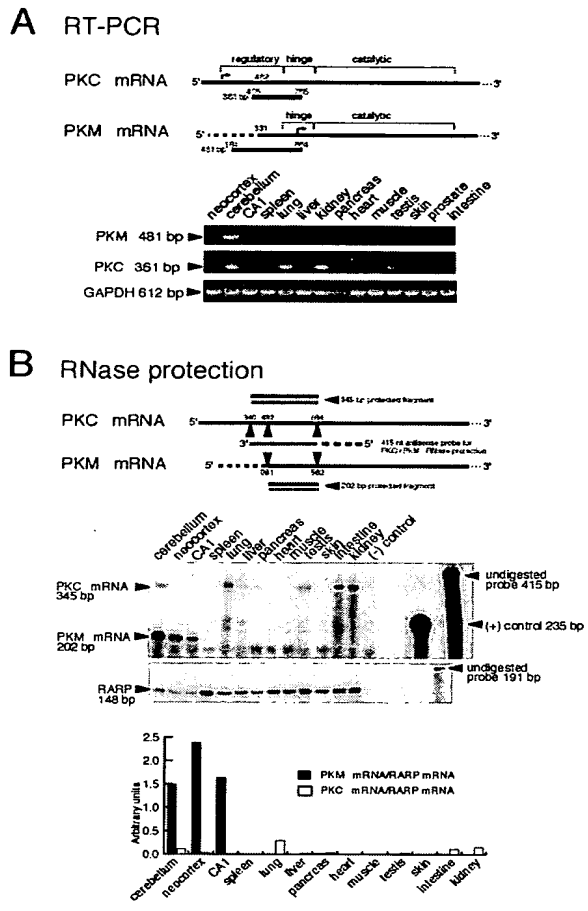

FIGURE 11

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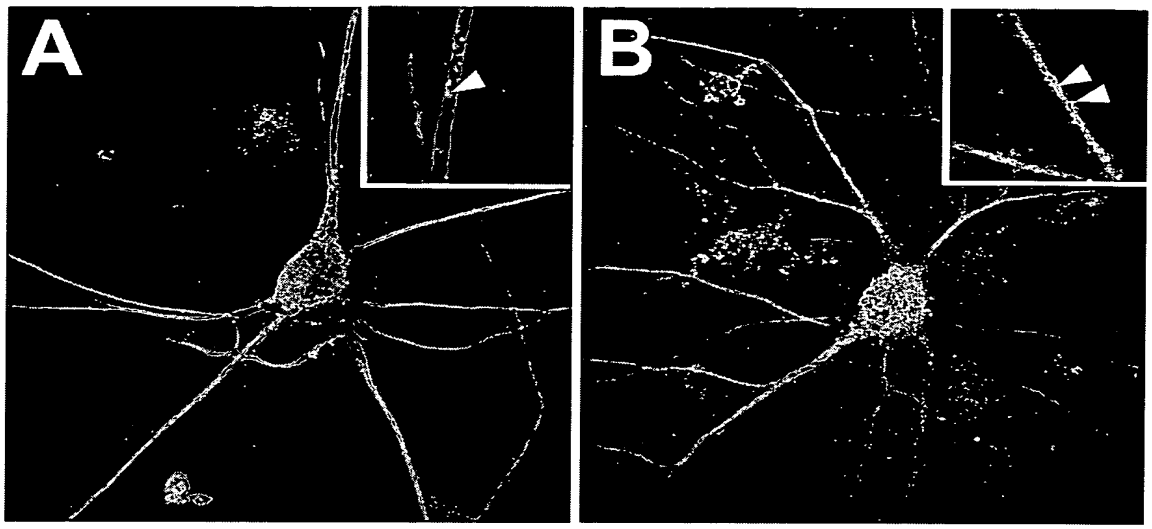

FIGURE 12

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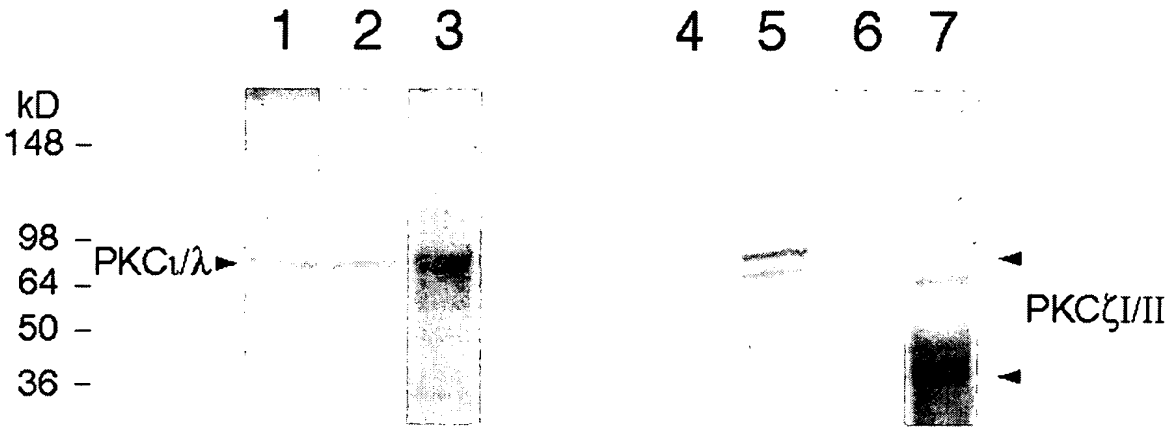

FIGURE 13

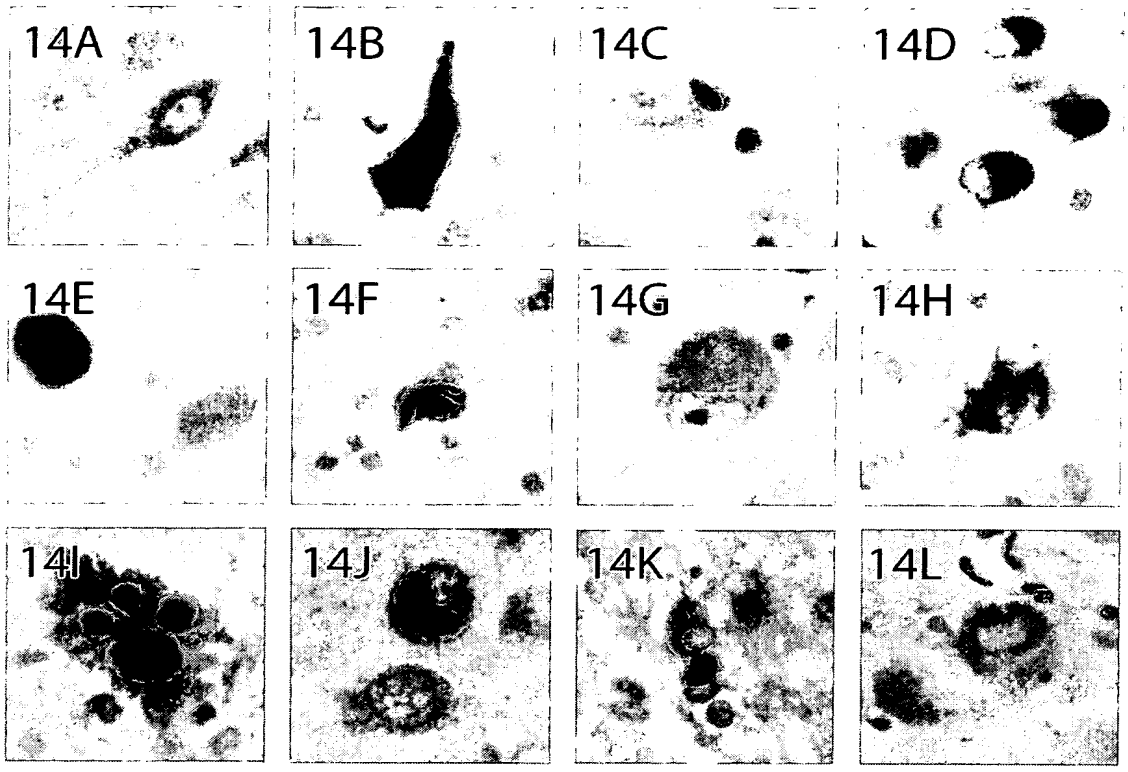

FIGURE 14

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